The Use of Lichens as Indicators of Ambient Air Quality in Southern Ontario

Patricia M. Tulumello

A thesis submitted to
The Department of Earth Sciences, Brock University
in partial fulfillment of the requirements
for the degree Master of Science

August, 2010
Brock University
St. Catharines, Ontario

Abstract

The inverse relationship between arboreal lichen species richness and sulphur dioxide in ambient air has been thoroughly documented in the literature. Previous work in southern Ontario has shown that lichen bioindication can identify areas of potential concern regarding air quality. The EMAN suite of lichens was applied in the City of Sarnia by surveying 458 Sugar Maple trees, in order to test the applicability of lichen bioindication under conditions of high mean SO_2 levels and high species richness values. The results of the survey were explored using Geographic Information Systems. A spatial relationship between lichen community variables, the Bluewater Bridge and the highway was identified. Lichen species richness, lichen percent cover and Index of Atmospheric Purity values were higher along the bridge and highway. No strong gradients were found between other known pollution sources and no lichen deserts were identified. The most common community grouping consisted of *Physcia millegrana* Degel, *Candelaria concolor* (Dicks) B. Stein, *Physcia aipolia* (Ehrh ex Humb.) Furnrohr; all of which are known nitrophytes. The relationship between substrate pH and lichen species richness was examined. Sites with a known source of anthropogenic chemical contamination were found to have a correlation of r^2=0.8 between lichen species richness and pH. The inverse was found for sites with no known source of contamination with a correlation of r^2=-0.72. The findings suggest that species richness may be influenced by altering substrate pH which promotes the growth of nitrophytic species capable of tolerating high SO_2 levels.

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1.0 INTRODUCTION

Lichens have long been used as biomonitors to assess ambient air quality. The disappearance of many lichen species from urban environments was noted as early as 1790, when Erasmus Darwin documented the extirpation of lichens downwind from smelters in northern Wales (Darwin, 1790). Many systematic studies over the next century directly linked the documented changes in the lichen communities to fluctuations in local air quality; in particular the burning of fossil fuels and sulphur dioxide (SO_2) emissions (Turner and Borrer, 1839; Grindon, 1859; Richardson, 1988). This highlighted the potential of lichens as primary indicators of changes in air quality. After the London smog event of 1952 which led to the death of four thousand people (Bates, 2002), mapping studies examined the spatial distribution of epiphytic lichen species across nations including Britain and the Netherlands while relating species distribution to differences in local air quality (Wit, 1983; Sloof and Wolterbeek, 1991; Carreras, Gudino and Pignata 1998). Several of these initial mapping studies (Sloof and Wolterbeek, 1991; Sloof and Wolterbeek, 2007) served as a baseline for the creation of national lichen monitoring networks in Britain and other European countries.

The European Union and its twenty-three members in the International Cooperative Programme on Integrated Monitoring of Air Pollution Effects (Van Herk, 2007; Ferreti, Brambilla, Brunialti, Fornasier, Mazzali, Frati, Santoni, Nicolardi, Gaggi, Brunialti, Guttova, Gaudino, Pati, Pirintsos and Loppi, 2003) have successfully established standard procedures for national air quality lichen monitoring systems, allowing for the sharing of data and information between countries.

Standardized lichen biomonitoring techniques are currently being used to estimate national pollution loads and to monitor the ecosystem response to both anthropogenic stressors and changes in air quality (Jeran, Jaimovi, Batic and Mavsar, 2002). In particular, lichen biomonitoring has been especially useful to interpret SO_2 levels in ambient air due to the sensitivity of many lichen species to SO_2 (Nash, 1973; van Dobben and ter Braak, 2007). This has enabled the participating countries to supplement air quality data from instrument networks.

In Canada, government agencies primarily use networks of chemo-mechanical sensors to track spatial and temporal changes in air quality (Environment Canada, 2007). However, dozens of short and long-term biomonitoring studies have been performed at the provincial level using moss, trees and agricultural crops as biomonitors to assess ambient ozone levels and to document damage to vegetation over a span of decades (*e.g.*, Ontario Ministry of the Environment, 2008; Glooschenko and Arafat, 1988). Few systematic lichen biomonitoring studies of air quality have been done in Canadian cities. One of the few Canadian studies that used biomonitors to assess air quality was 'An Investigation of the use of lichens and mosses as biomonitors of acidic precipitation in Ontario' by the Ontario Ministry of the Environment (MOE) to monitor the impact of ozone and acid rain over a span of decades (Ontario Ministry of the Environment, 1990). In the past few years, Environment Canada's Environmental Monitoring and Assessment Network (EMAN) created a lichen monitoring protocol to monitor ambient air in Canada. EMAN (Environment Canada, 2008) recommends the use of nineteen arboreal lichens commonly found in the Canadian mixed-hardwood forest zone for biomonitoring

studies. The purpose of this protocol (EMAN, 2007) was to provide amateur scientists with a tool that could be used to identify areas where air quality or site disturbances have altered lichen communities. This protocol was first tested in Hamilton, Ontario where McCarthy, Craig and Brand (2009) showed that the EMAN recommended protocol can be used to identify variations in air quality within a city. The study was followed by research that examined lichen species richness on maple trees adjacent to 14 of the Ontario MOE SO_2 monitoring sites. A strong correlation (r^2=0.81) was found between the mean annual SO_2 levels and the number of lichen species at the stations (D. McCarthy, personal communication, June 2006).

In 2006, D. McCarthy undertook lichen species richness surveys at three industry-controlled (owned and operated by the Sarnia-Lambton Environmental Association) air quality monitoring sites in Sarnia. When these Sarnia data were plotted alongside data from the other sites, Sarnia had higher species richness values than expected (Figure 1.1). The Sarnia data not only appear anomalous, but also seem to contradict an extensive body of literature that has reported an inverse relationship between lichen species richness and SO_2 levels in ambient air (*e.g.*, Nash, 1973; McCune, 1988). To be able to use the EMAN approach to effectively identify areas of concern, it is necessary to explore why high lichen species richness has been found in Sarnia despite high mean annual SO_2 levels.

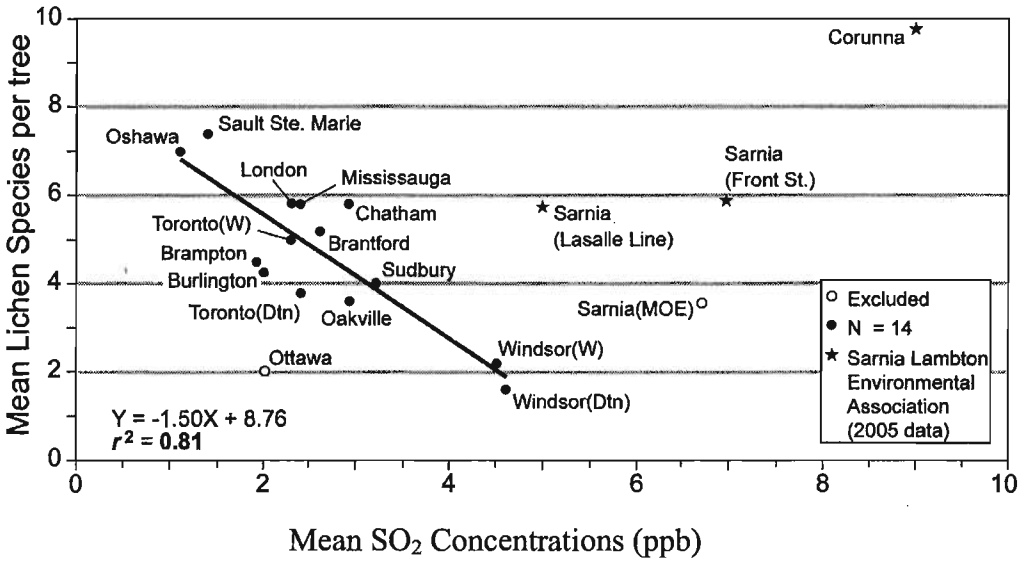


Figure 1.1 Lichen species richness and mean SO_2 at the 14 MOE AQI sites (D. McCarthy, personal communication, June 2006).

1.1 Research Objectives

This study explores the use of lichen monitoring as a method to identify areas of concern linked to ambient air quality across the City of Sarnia. The methods selected for this study are those that have been widely applied in both Europe and North America, but have not seen wide application in Canada and have the greatest potential for inexpensive, non-destructive biomonitoring of air quality (Gombert, Asta and Seaward, 2004; van Herk, 2007).

Emphasis will be placed on the development and application of an approach that integrates measures of habitat disturbance using Geographic Information Systems and preliminary assessment of bark pH with changes in the lichen population. While such studies as van Herk (2007) have shown that the pH of bark has potential as a biomonitoring technique as it appears to affect lichen community composition (van Herk, 2007), there is not yet a standardized protocol for bark pH analysis. Thus,

replication, inter-study comparisons and evaluation of bark pH data are problematic. Nevertheless, the preliminary assessments of tree bark pH presented in this thesis, might allow future workers to identify gross differences in pH that can serve as a useful supplement to the EMAN approach to lichen monitoring. Consequently, bark pH is another aspect of this study.

This research has three core objectives: (i) to apply current lichen biomonitoring protocols applicable to Ontario's urban environments; (ii) to apply alternate lichen biomonitoring approaches that can be used to assess whether the Index of Atmospheric Purity (LeBlanc and DeSloover, 1970) values accurately reflect differences in ambient air quality in Sarnia; and (iii) explore the potential connections between the pH of tree bark, microhabitat and lichen community composition in order to determine whether any of these variables may be influencing lichen species richness in Sarnia.

1.2 Scope of Research

This study reviews and applies lichen biomonitoring techniques to assess ambient air quality in Sarnia, Ontario. The main geographical focus is the City of Sarnia, but limited sampling has also been performed in Windsor and Hamilton to permit inter-city comparisons of lichen species richness and tree bark pH in order to further explore any possible relationship.

This research developed and used various maps and indices to describe and search for spatial differences in Sarnia's lichen communities. Community groupings and their spatial distribution were identified. It also statistically explored the link between Sarnia's lichens and their environment. This included attempts to develop a

preliminary description of bark pH as it relates to lichen abundance at sites in Sarnia, Hamilton and Windsor.

2.0 LITERATURE REVIEW

2.1 Introduction: Lichens as Bioindicators

Lichens are found in all biomes and most habitats from rural to urban (Nash, 1996). They can be found on a wide range of substrata including ground (terricolous lichens), water (aquatic lichens), on rocks or human-made structures (epilithic or saxicolous lichens) and on tree bark (corticolous or epiphytic lichens; Richardson, 1992; Nash, 1996). Owing to the large number of habitats that lichens can tolerate, they can often be found in high numbers even when higher plants and other organisms are absent (Richardson, 1992). Lichens are often among the first organisms to colonize recently disturbed sites and in some instances they may form semi-permanent "climax" populations which may last for centuries (Richardson, 1992). Because lichens are perennial, they can be used for biomonitoring studies throughout the year and lichen community variables, such as lichen species richness, can be linked to mean annual contaminant levels. Several studies (*e.g.*, Ferry, Baddeley and Hawksworth, 1973; Case, 1980; Addison and Puckett, 1980) have demonstrated that lichens have a quicker response and higher sensitivity to contaminants than higher plants.

Lichens can be used to provide an integrated measure of most ecological stressors including changes in habitat conditions and dust accumulation. Epiphytic lichen species often have higher accumulation rates of contaminants from airborne particles and precipitation than higher plants and even mosses (Larson, 1987). This sensitivity is partly a function or a consequence of lichen physiology and the chemistry of the thallus and the surrounding environment (Richardson, 1988). Lichens are passive

absorbers of air and waterborne materials as they lack stomata and a waxy cuticle to protect them (Richardson, 1992). As a result, both nutrients and contaminants in particulate form can accumulate in the lichen through dry deposition. Contaminants can also enter lichen tissue through ion exchange (Adamo, Giordano, Vingiani, Castaldo Cobianchi and Violante, 2003). The extent of the influence of substrate chemistry on the ability of lichens to trap and accumulate contaminants is not fully known. However, many studies (*e.g.*, Sloof and Wolterbeek, 1993; Kurina and Vitousek, 1999; Schmitt and Slack, 1990; Pharo and Beattie, 2002) have found a link between certain lichen species and certain substrate types. Variables such as distance from emission source (Dubey, Pandey, Upreti and Singh, 1999), exposure intensity and duration (Adamo *et al.*, 2003), habitat (Bargali, Castello, Gaspero, Lazzarin and Tretiach, 1992), prevailing winds (Evans and Hutchinson, 1996) and the element or compound itself all influence the rate of accumulation (Adamo *et al.*, 2003). Clearly transfers between substrate and lichens can readily occur due to surface washing as well as erosion and airborne dust (Adamo and Violante, 2000). Many studies have found greater concentrations of elements in the lower thallus and rhizinae, the sections of the lichens closest to the substrate in both corticolous and epiphytic lichens (Goyal and Seaward, 1982a; Branquinho, Brown and Catarino, 1997).

There are three broad categories of studies that use lichens to assess ambient air quality: lichen population measures, chemical analysis of lichen tissue and lichen thalli damage assessment. Lichen population measures have been widely used in both Europe and North America and have been the preferred method for the establishment of national lichen monitoring systems (McCarthy *et al.* 2009; Zechmeister and

Hohenwallner, 2006). Lichen population measures are based on lichen population information such as species richness and weighted indices based on percent cover. The Index of Atmospheric Purity (IAP, LeBlanc and De Sloover, 1970); the Index of Poloeotolerance (IP, Trass, 1973) and the Lichen Diversity Value (LDV, Asta, Ernhadt, Ferreti, Fornasier, Kirschbaum, Nimis, Purvis, Pirintsos, Scheidegger, Van Haluwyn and Wirth, 2002) have all been developed from this approach. When used as a primary assessment technique for air quality, lichen population measures can be statistically analyzed and correlated with data from other sources. The use of GIS to examine any trends in the data is becoming more common (*e.g.*, Johansen, Tommervik, Guneriussen and Pedersen, 1994; McCarthy *et al.*, 2009). Lichen population measures allow the inference of long term ambient air quality trends, as seen by the European monitoring networks. They can only broadly characterize air quality as lichens respond to a variety of stressors. Limitations of this approach are the lack of standardized sampling procedures and the difficulty of identifying small lichen thalli to species level in the field (Kinnunen, Holopainen and Karenlampi, 2003).

Chemical analysis of lichen tissue has been another common approach in biomonitoring studies. Lichens are able to absorb almost every element in the periodic table (Garty, 1993) and many studies have correlated the chemical content of lichen tissue with pollution levels (*e.g.*, Bennett and Wetmore, 1997; Fenn, Baron, Allen, Rueth, Nydick, Geiser, Bowman, Sickman, Meixner, Johnson and Neitlich 2003). Occasionally, workers use rare earth elements or isotopes sampled in lichen tissue to track contaminants to a point source that has a known chemical signature

(*e.g.*, Simonetti, Gariepy, Banic, Tanabe and Wong, 2004). Chemical analysis has rarely used in conjunction with lichen population measures such as lichen species richness and lichen percentage cover, although such studies are becoming more frequent. Approaches vary depending on the conditions of the site with some studies collecting homogenized samples from many thalli (*e.g.*, The United States Forest Inventory and Analysis National Program, 2008) and others using particular sections of the thalli (*e.g.*, Prussia and Killingbeck, 1991). Many crustose and foliose lichens have a radial growth form, where the oldest tissue is found in the central part of the thallus while the youngest is found at the fringes. Unfortunately, it is difficult to estimate accurately the age of the lichen and its exposure time to pollution (Richardson, 1992). The U.S. Forest Lichen Monitoring Program chemically analyzed several lichen thalli in a homogenized sample for each forest in order to record spatial and temporal changes (Wetmore, 1989). Other studies chemically analysed the substrate, such as bark (*e.g.*, Gustafsson and Eriksson, 1995; van Herk, 2007) rather than lichens.

Some lichen biomonitoring studies involve lichen thalli damage assessment. This method typically involves the measurement and assessment of various parameters related to the integrity and functions of the lichen photosystem and is generally used to quantify damage to the lichen thallus. Electrical conductivity (Shirazi, Muir and McCune, 1996), CO_2 exchange (Scheidegger and Schroeter, 1995), Pulse Amplitude Modulated (PAM) Fluorometry (Stibal, Elster, Šabacká, and Kaštovská, 2007) and chlorophyll degradation (Gonzalez and Pignata, 1994) may be used to assess the vitality of the lichen. This approach often monitors changes in lichens transplanted to

areas with air quality concerns. However, it is sometimes difficult to attribute metabolic changes in lichen transplants to a source or timeframe.

Despite the documented usefulness of lichen population measures, chemical analysis and lichen thalli damage assessment in assessing air pollution, lichen population measures are the most widely used. When applied they can provide a non-destructive, preliminary assessment of air quality over a large area and the data collected can be correlated with instrumental data (Von Arb, Mueller, Ammann and Brunhold, 1990; van Dobben and ter Braak, 2007). Lichen population data can be analyzed using GIS (*e.g.*, Iverson and Prasad, 1998; McCarthy, 2009). GIS is a multiscale technology which can be used to detect changes in lichen abundance data (Brabyn, Green, Beard and Seppelt, 2005). It is being used in an increasing number of studies in place of conventional mapping techniques.

With lichen biomonitoring and bioindication in general, sampling protocols and techniques may have to be modified to accommodate site specific differences (*e.g.*, a lack of suitable substrates). As with many other biomonitors, it is often difficult to determine the variable(s) affecting a population. This problem is common in urban areas where many contaminants may be present (Kranner, Beckett and Varma, 2002). A combination of several contaminants or habitat variables may be affecting the lichen population resulting in a lack of correlation between lichen monitoring data and a specific contaminant.

2.2 History of Lichens as Bioindicators

The use of lichens as bioindicators began in Europe when it was noted that diversity and the percent cover of arboreal lichens decreased with increasing

emissions from industrialization and the subsequent burning of fossil fuels. This was first documented near smelters in Wales by Erasmus Darwin in 1790. Publications soon emerged that represented early attempts to better define the link between lichens and air pollution and propose that lichens could be used as air quality indicators (Gridon, 1859). However, it was Nylander (1866) who first proposed that lichens might be used as indicators of air quality. This hypothesis was soon examined in other studies through the use of surveys with Johnson (1879) producing the first publication on lichen diversity and air quality downwind of collieries.

Information on the relationship between the spatial distribution of lichens and air quality, such as the findings of the study by Johnson (1879) led to the development of zonal scales. Sernander (1926) identified three distinct zones of lichen species richness in and around Swedish urban centers. Areas devoid of lichens, generally located in the inner city were termed 'lichen deserts'. A secondary zone with fewer species than areas outside of the city was called a 'struggle zone', while a 'normal zone' with greater lichen diversity was found outside of the cities.

The use of zonal scales of lichen diversity and vitality was tested in 1952, when London experienced a smog event severe enough to cause an estimated four thousand premature deaths and an additional eight thousand deaths in the following weeks (Bates, 2002). The government created a network of air quality monitoring devices for smoke and SO_2. Approximately 1300 gauges were distributed nation-wide in both urban and rural areas making it possible to correlate lichen community characteristics with air quality data (Gilbert, 1965). By the early 1970s, a scale had been created that

estimated the sensitivities of lichen species to mean SO_2 levels in ambient air (Hawksworth and Rose, 1970; Hawksworth, 1973).

This scale enabled further studies to correlate their findings with the mean annual winter SO_2 concentrations in ambient air (Rose, 1970). Gilbert (1974) conducted a field survey of lichen species. The collected data were then categorized to create six zones which were intentionally simple to identify so that school children could participate in the national survey. The zonal scale assisted in map generation by enabling spatial trends to become visible. Subsequent work showed that this zonal scale clearly reflected ambient SO_2 and lichen zonations in other European centers (Skye, 1958; McCune, 1988). The results of the Rose (1970) survey allowed for the creation of maps and the classification of both large (within a country) and small scale zones (within a city) based on topographic maps, of lichen diversity and ambient air quality in England and Wales. Another landmark study on lichens and air quality was performed by Gunn (1996). This study documented the re-establishment of lichen species in an area of approximately 7 km^2 which was previously devoid of lichens (a 'lichen desert') near Sudbury, Canada. The study suggested that the site's previous lack of lichens was due to high concentrations of emissions, such as SO_2 from smelters.

2.2.1 Species Tolerance Levels and SO_2

An inverse relationship between lichen species richness and SO_2 levels was documented in various studies, throughout the 1950s and 1960s (e.g. Skye, 1958). Pearson and Skye (1965) and Rao and LeBlanc (1966) tested lichen survival by fumigating lichens in enclosed flasks with SO_2. Hill (1971) tested the tolerance levels

of species that displayed varying tolerances in the field to a solution of sulphite ions. The results were statistically similar to those found in fieldwork, in which increasing levels of SO_2 resulted in a decrease of lichen vitality and ultimately death. However, not all experiments returned good correlations and a few discrepancies became apparent. Nash (1996) addressed this problem by using Spearman's rank correlation coefficients in conjunction with the data collected by Hawksworth and Rose (1976). He found that the Hawksworth and Rose (1976) scale is representative for SO_2 tolerance, but not for all data sets. Nash suggested that these discrepancies were caused by the nature of the laboratory study. For example, lichens studied in the field are often exposed to fluctuating concentrations over a significantly long time period (years) compared to laboratory studies (hours). Hawksworth, Coppins and Rose (1976) suggested, based on the lichen distributions found in their 1975 survey, that other factors in addition to SO_2 influenced lichen distribution. A previous study by Hawksworth and Rose (1974) supported this by identifying microclimate as a contributing factor to different intraspecies tolerance levels between populations in an oceanic microclimate and those in a continental one. The most probable cause for this is the different length of time in which the communities were physiologically active in each year (Hawksworth and Rose, 1974).

A different method was used by Zakshek, Puckett and Percy (1986) to assess the potential benefits of lichen biomonitoring studies by using the fruticose lichen, *Cladina rangiferina (L.) Harm.* to determine pollution levels in different locations. Lichens were collected from 14 sites that ranged throughout eastern Canada using a sampling grid of 127 km^2. The results of the chemical analysis of the lichen tissue for

lead (Pb) and sulphate (SO_4^{2-})values were compared to the results found by Puckett (1978) who collected lichens from sites scattered around the North West Territories within a sampling grid of a similar size. The results showed the highest concentration of contaminants in terricolous and arboreal lichens collected from central and north-central Ontario with a correlation between deposition rates of airborne Pb and SO_4^{2-}. Zakshek *et al.* (1986) found that higher chemical concentrations in lichen tissue appear to not only be influenced by deposition, but were also accumulated by rainsplash and local sources of lead and sulphate. The use of epiphytic lichens that exist higher than 0.5 m on the trunk of a tree could minimize exposure to rainsplash in future studies. Despite the use of both terricolous and arboreal lichens, these studies can still provide insight into the general relationship between lichens and contamination. However, caution is required when using such data as a baseline for future studies since the terricolous lichens may have higher concentrations due to rainsplash than the arboreal species.

2.2.2 Lichens and pH

Several studies have shown that pH may influence lichen species richness and the ability of a lichen to photosynthesize and the uptake various contaminants. A study by van Herk (2007) examined the relationship between bark properties (pH, electrical conductivity, NH_4^+, SO_4^{2-}, NO_3^-) and levels of air pollution (SO_2 and NH_3) and lichen community composition. In particular, the study focused on the relationship between the abundance of nitrophytic lichen species, which are lichen species that often inhabit and sometimes thrive in locations that may have poor air quality, high levels of nitrogen and basic substrate pH. Nitrophytic species are found to have the

greatest percent cover on trees with a bark pH between 5 and 7 and are generally not found on tree species with more acidic bark with pH values below 5 (Barkman, 1958). One hundred and four lichen monitoring sites were established across the Netherlands. Ten wayside *Quercus robur* trees with lichen cover were surveyed per site. Bark samples were collected from seventy-six lichen monitoring sites ranging from rural to urban. The dried bark samples were ground and 5 g of each sample were suspended in 50 mL of distilled water. Each suspension was then shaken for an hour, given a settling period of a day and then shaken again for an hour and centrifuged. A digital measuring instrument with a glass electrode was used to measure pH, NH_4^+, SO_4^{2-} and NO_3^- were measured using a spectrophotometer. That study found that bark pH and the tolerance levels of the individual lichen species to various contaminants were found to be the primary influence on lichen community composition in the Netherlands. These two factors were found to be independent of each other with bark pH exerting a much greater influence on the presence of nitrophytic lichen species than the concentrations of various contaminants, including SO_2 levels. In particular, that study found that the increase in the number of nitrophytic species and the decrease of acidophytic species, which appear to be sensitive to SO_2 levels, were linked to an increase in bark pH which occurred over the span of a decade (van Herk, 2007). A study in Finland by Kuusinen (1994) found that one of the factors that contributed to low lichen species richness values was a high average bark pH. This study compared the bark pH of samples with low lichen species richness to those with high lichen species richness and found that average high bark pH values were associated with high lichen species richness. The lichen

species indentified in that study were predominantly acidophytes, which were found to decrease with higher bark pH values in the study by van Herk (2007). That study identified species such as *Lobaria pulmonaria,* which is an acidophtyic member of the EMAN suite. Bark pH may be found to have a stronger correlation with lichen species richness in other urban areas that have experienced changes in SO_2 levels in addition to the areas studied in Finland and the Netherlands. The rate of change of bark pH may be slower than the rate of change of air quality. Bark pH may assist in partially explaining causality for the spatial distribution of lichens and lichen species richness in urban areas, which have experienced a decline in SO_2 levels. However, further study would be required to assess what factors are influencing bark pH.

Bark pH may also influence the vitality and functions of the lichens. It is possible that the pH of the bark and of water (in the form of precipitation and run-off over the bark) to which the lichens are exposed may alter the efficiency of the lichen's uptake of various contaminants. Turk and Wirth (1975) examined the influence of pH on the absorption of SO_2 by the lichens *Xanthoria parientina* and *Hypogymnia physodes* under laboratory conditions. The severity of damage was found to increase with decreasing pH levels. The study concluded that the differing levels of damage to the lichens was a function of the concentration of the 'toxic products' generated from the reaction of SO_2 with water, which was dependent on the pH; lichens accumulate more contaminants during the process of wet deposition which would be common in nature (Turk and Wirth, 1975). Hass, Bailey and Purvis (1998) found that the absorption of uranium by *Peltigera membranacea* varied with exposure to solutions with different pH levels. The highest rate of absorption was found to occur at a pH of 4.5. Higher

pH levels led to less absorption (Hass *et al.* 1998). The differing levels of damage to different lichen species by pH may be linked to the spatial distribution of lichens and lichen species richness. Lichens that may be expected to be found based on the air quality of the study area may not be present due to bark pH levels.

2.2.3 Lichens and the Index of Atmospheric Purity

Zonal scales relating lichen presence to ambient air quality were widely used throughout Europe and their successful application soon led to a call for the development of a method for quantitative assessment of ambient air quality using lichens (Kricke and Loppi, 2002). The Index of Atmosphere Purity (IAP) was formulated by LeBlanc and DeSloover (1970) and is perhaps the most commonly used equation. Since it was first used to relate lichen diversity to ambient air quality, it has since been widely adapted in order to avoid variations in lichen diversity due to factors other than air quality. At least eighteen different IAP equations have been presented in studies, although many are no longer commonly used (Kricke and Loppi, 2002). The IAP equation selected for this study is still commonly used in the literature (*e.g.*, McCarthy *et al.*, 2009).

$$IAP = (\sum_{i=1}^{n} Q_i \, x f_i)/10$$

Equation 2.1 The Index of Atmospheric Purity (IAP), (McCarthy, 2005). In equation 2.1, the Index of Atmospheric Purity (McCarthy, 2005), n = the number of species of lichens found at a given tree, Q = the ecological index of a lichen species, and f = the frequency of coverage for that particular species at the individual tree.

The Index of Atmospheric Purity (IAP) is a quantitative method that uses the presence, absence and abundance of lichens in order to generate an air quality index. This index works by generating values that are meant to be reflective of the ambient air quality based on lichen diversity.

The calculation and interpretation of IAP values has been successfully used to assess ambient air quality in many studies (*e.g.*, Jeran *et al.*; 2002, Calvelo and Liberatore, 2004; Gustav-Zechmeister and Hohenwallner; 2006, Krommer, Zechmeister, Roder, Scharf and Hanus-Illnar, 2007; McCarthy *et al.*, 2009). The IAP equation was based on the findings of Skye (1958), Gilbert (1974) and Hawksworth and Rose (1970). That work found that SO_2 was the primary factor responsible for declining lichen diversity in urban areas. LeBlanc and DeSloover (1970) calculated IAP levels in a 563 km^2 study area centered on the City of Montreal, Canada. Ten trees of varying species including *Ulmus Americana* (American Elm) and *Acer saccharum* (Sugar Maple) were selected at each of their 349 sites. Sites were selected to be as ecologically similar as possible in order to decrease natural variation caused by topography, etc. Only mature, isolated trees in fields or by the roadside with similar diameters and bark properties were selected. The IAP was calculated for each site and mapped using five intervals. The general trend found in this study was that IAP increased with increasing distance from urban areas. LeBlanc and DeSloover (1970) did note that there were a few small areas of the map in which the IAP values contrasted greatly with surrounding areas which could not be explained. This indicated that IAP may be a useful tool to view large gradients, but may be influenced on the small-scale by unknown factors such as local contamination or disturbances. Although air quality monitoring data were not reported in this study it was implied

that spatial differences in lichen species richness was an indicator of ambient SO_2 levels which had been described by LeBlanc (1969).

Case (1980) produced IAP values for a 35 km^2 area surrounding three sour gas processing plants near Fox Creek and Whitecourt, Alberta. IAP values, average sulphation rates and sulphur content were statistically analyzed. The results of that study indicated that arboreal lichens primarily uptake sulphur from the atmosphere and that IAP could be applied to successfully indicate zones of air quality concern. However, a subsequent study by Granger (1970) compared air quality monitoring data against the IAP values calculated by LeBlanc and DeSloover (1970) and found no clear relationship. That study suggested that other factors were contributing to differences in lichen distribution such as the relationship between lichen distribution and the length of exposure to contaminants and the concentration of the contaminants. Case (1980) also tested the application of IAP values to monitor the average annual SO_2 levels. A total of 99 ecologically similar study sites were selected and 10 trees of the genus *Pinus* were selected at each. A total of 60 lichens ranging from sensitive to insensitive to air pollution were identified, although many of these were present only at a few sites. The study found that lichen species richness and IAP values increased with increasing distance from a known source of pollution, like Granger (1970), Case also found a poor correlation between IAP values and SO_2 levels ($r^2 = 0.13$). Case (1980) later addressed this problem by noting that due to contaminant accumulation within lichen tissue, the IAP value does not represent the fumigations of a single year, but is more indicative of long-term trends. Since the IAP really is a weighted measure of the relative abundance of lichen species in a sample set, the link between IAP and

any one stressor is entirely unknown. Thus, causality is not measured or assigned by the calculation of IAP values. Case (1980) reported that lichen species richness and IAP values in his study were low where ambient SO_2 levels were high and IAP values dropped with increasing distance from the source, but noted that this does not directly imply causality because other factors may have influenced the IAP values.

As Case (1980) and others have demonstrated, if long-term ambient air quality has had a negative impact on the colonization and survival of lichens, then it is possible that the IAP values might be clearly related to one or more of the contaminants found in ambient air. However, lichen communities are not always in balance with ambient air quality. Thus, IAP should be interpreted as a general measure of stress loads and disturbance regimes over the longer term. Presumably the IAP approach is at least as effective an indicator of ambient air quality as other species richness approaches (*e.g.*, Asta *et al.*, 2002). Those widely used approaches, which include lichen species richness and percent lichen cover, do not assign causality and do not assign relative weights to account for the rarity of some species (Gustav-Zechmeister and Hohenwallner, 2006).

Several studies have called attention to the limitations of the IAP approach. For example, Herben and Liška (1986) examined the Q variable in the equation and considered its influence on the validity of the results. They noted that Q can be influenced by the number of pollution sensitive species, the frequency and the distribution of species, the total number of species and the number and distribution of study sites. Gombert, Asta and Seaward (2003) in Grenoble, France found that IAP was not capable of distinguishing local sources of pollution such as local pollution

plumes and roads. Several studies have suggested that vehicle emissions may influence lichen abundance by creating conditions favourable to the establishment and growth of nitrophytic species (Case, 1980; van Herk, 2007). It has been suggested that since the IAP equation was developed at a time in which the majority of studies found a strong correlation between SO_2 and lichen diversity, it may not be accurate for urban areas; which have experienced a decline in SO_2 (Gombert *et al.*, 2003). Despite modifications to the IAP equation, Gombert *et al.* (2003) found that environmental variables were still able to influence lichen distribution. Furthermore, it was found that sites dominated by either nitrophytic or acidophytic species (lichen species that tolerate more acidic substrate) tended to skew the IAP results.

2.2.4 Lichens and Decreasing SO_2 Levels

Studies involving the use of lichens to assess ambient air quality during the 1960s and 1970s were conducted during a time in which SO_2 levels had increased or remained stable for many years (Kricke and Loppi, 2002). Sulphur dioxide levels began to decrease in the late 1970s due to stricter emission controls and new technology (Kricke and Loppi, 2002). The decrease in SO_2 levels in many urban centers began to allow for their recolonization by lichen species that had not been present under higher SO_2 levels.

As SO_2 levels in ambient air begun to decline in the 1980s, Hawksworth and McManus (1989) found that not all lichens uniformly recolonized the City of London, England. In some locations, recolonization of lichen species was uneven and lichen species invaded areas where air quality was still relatively poor. Fifty sites were surveyed and it was found that species expected to be present under lower SO_2 levels

had recolonized the area, while other species which had been expected to be present under moderate SO_2 levels were not. This was termed "zone skipping" (Hawksworth and McManus, 1989). Gilbert (1992) later expanded this concept with the introduction of the term 'zone dawdlers' to describe species that took longer than expected to recolonize areas.

Clearly, it is difficult to correctly predict lichen recolonization of urban areas which have undergone a decrease in SO_2 levels in ambient air. There are many factors believed to influence recolonization. Factors such as the type of lichen, the method of reproduction, the location and health of the lichen, substrate characteristics and wind direction and frequency may all influence recolonization. A study by Fox (1999) found that fruticose and foliose lichen species, which are capable of reproducing asexually were more likely to successfully recolonize an area than crustose species. This study also found that of the fruticose and foliose species, those that thrive on neutral or nutrient-enriched bark appeared to have been more successful at recolonization (Fox, 1999). A second factor is the number of genotypes of the lichen species that are recolonizing. Crespo, Bridge, Hawksworth, Grube and Cubero (1999) examined three genotypes of long-established and recolonizing *Parmelia sulcata* communities from the UK and Spain in order to determine whether all genotypes were equally active in recolonization. They found that only one genotype was responsible for recolonization in the study area (Crespo *et al.*, 1999). Chance is another important factor in recolonization. The introduction of lichen propagules into the recolonization zone is based on the chance that the wind, birds or another method of transportation will bring the propagules into the zone. These factors and possibly

others which are still unknown result in difficulty in predicting lichen recolonization patterns.

2.3 The History of Air Pollution in Sarnia

Sarnia is located in south-western Ontario, with Lake Huron as a boundary to the north and the St. Clair River and Michigan to the west. Approximately 70,000 people reside within its boundaries which contains an area of almost 161 km^2 of land. Due to its proximity to Michigan, Sarnia is one of the busiest commercial border crossings in the world (The Corporation of the City of Sarnia, 2007).

Sarnia has only two air monitoring stations operated by the Sarnia Lambton Environmental Association, with a third located nearby in Corunna, to monitor SO_2, NO_x, NO_2 and particulate matter. Two other air quality monitoring stations are operated in Sarnia by the Ministry of the Environment (MOE). With so few monitoring stations, there is a risk that the data collected from the stations may not be representative of the air quality of the entire city. The two air quality monitoring stations in Sarnia are located on Front Street and LaSalle Line and the highest wind speeds are consistently reported at Front Street due to the open nature of this location. LaSalle Line experiences the greatest number of low wind speed (or calm) conditions each year mostly due to the protected nature of the site. The Front Street location consistently reports higher concentrations of SO_2 than the LaSalle Line station (Sarnia-Lambton Environmental Association, 2006).

Sarnia has the highest reported concentration of SO_2 in Ontario as seen in Figure 2.1. In 2006, Sarnia had a reported annual mean concentration of 8.3 ppb of SO_2 compared to a provincial average of 2.64 ppb (Ontario Government, 2007).

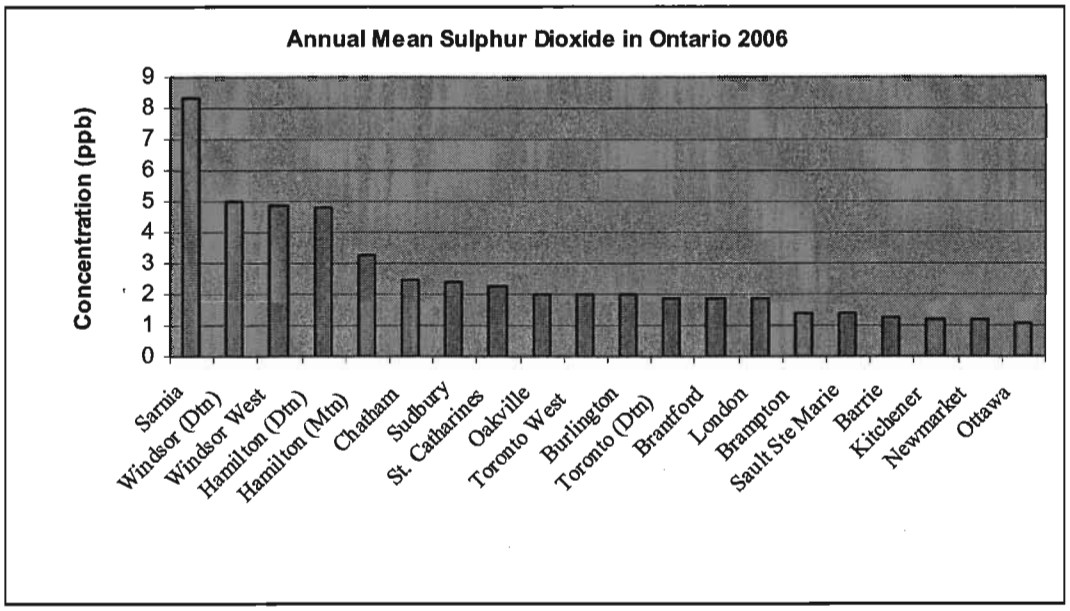


Figure 2.1 Annual mean SO_2 concentrations in Ontario cities for 2006 (Ontario Government, 2007).

Although the heavy border traffic contributes, a greater percentage of the SO_2 is emitted from the petrochemical industry located in Sarnia (The Ontario Government, 2007). The petrochemical industry in Sarnia is approximately 40% of Canada's chemical industry (The Corporation of the City of Sarnia, 2007). Forty-six facilities that are located within 25 km of Sarnia are registered under the National Pollutant Release Inventory (NPRI, 2006). On the American side of the border, but still located within 25 km of Sarnia, there are sixteen facilities registered under the U.S. Toxic Release Inventory (TRI, 2006). Some of the emissions from these facilities are designated criteria air contaminants such as SO_2, CO, NO_x, total particulate matter, particulate matter equal to or less than 10 microns (PM_{10}), particulate matter equal to or less than 2.5 microns ($PM_{2.5}$) and volatile organic compounds (VOCs). Many of these chemicals can cause phytotoxic damage.

As seen in Figure 2.2, mean annual sulphur dioxide levels have decreased in the Sarnia area. Despite a high contaminant concentration rating, compared to other Ontario cities, contaminant concentrations in Sarnia have declined over the last decade (National Atmospheric Deposition Program, 2007, Sarnia-Lambton Environmental Association, 2006). Much of this decrease is due to the use of new technology (*e.g.*, scrubbers in smoke stacks) and stricter regulations and enforcement (Sarnia-Lambton Environmental Association, 2006).

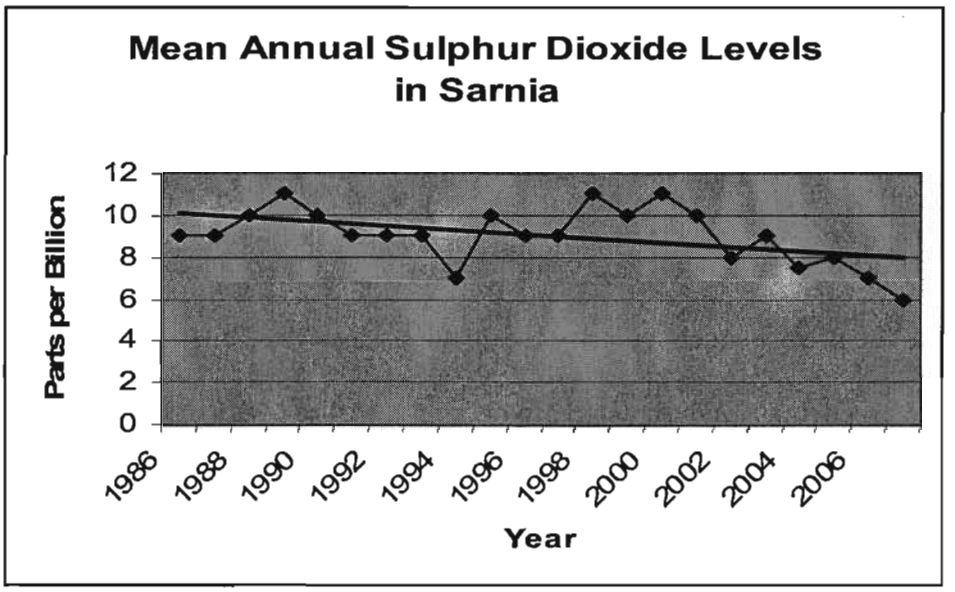


Figure 2.2. Mean annual sulphur dioxide levels in parts per billion recorded in Sarnia (Sarnia-Lambton Environmental Association, 2006).

Despite the decrease in average deposition levels, fumigation events still occur several times each year (Sarnia-Lambton Environmental Association, 2006). Apart from having an impact on human health, fumigation events can significantly raise the amount of phytotoxins at ground level and might influence lichen species richness (Zambrano and Nash, 2000). A total of 13 recorded releases of levels of contaminants high enough to be reported to the Ministry of the Environment and 144

alerts of high levels of SO_2 lasting an average of 17 hours occurred in Sarnia between 1982 and 2003 (Sarnia-Lambton Environmental Association, 2006). Since the spatial extent and the concentrations of SO_2 at various locations aside from the four air quality monitoring stations in Sarnia during these fumigation events are unknown, lichen surveys may assist in determining the extent of these fumigation events.

3.0 METHODS

3.1 Lichen Surveys

Two lichen surveys were conducted. The first survey (in early June, 2006) served as a preliminary test of the sampling protocols and the lichen identification key. The second survey (in early October, 2006) was used to create a denser network of survey points and generate data that could be used to better describe and statistically characterize the habitats, substrates and lichen communities.

3.1.2 Site and Tree Selection

Sarnia was divided into sixty-three 1 km^2 sampling plots based on a 1: 25, 000 scale map. The initial survey in June of 2006 examined four mature *Acer* trees in each of the sixty-three 1 km^2 plots. Only mature, undamaged, open grown trees that had lichen cover similar to others nearby were selected. The trees had upright trunks that measured >76 cm in circumference at chest height. Each tree was at least 5 m from any obvious sources of artificial nitrification (*e.g.*, a compost pile, a planter box, etc.), with the exception of streets since vehicles contribute directly to air quality through their emissions. Their locations were identified using GPS and the locations, tree diameter and lichen community variables were recorded for each tree.

The second survey was conducted in the same 63 km^2 area as the initial survey. In the second survey, eight mature, open grown, upright *Acer saccahrum* (Sugar maple) trees were sampled per site, but only trees with a diameter greater than 64 cm at chest height were surveyed. Tree selection in this survey was done using a random

sampling design. None of the trees sampled in the initial survey were selected for the second survey. The location of the survey sites can be seen in Figure 3.1.

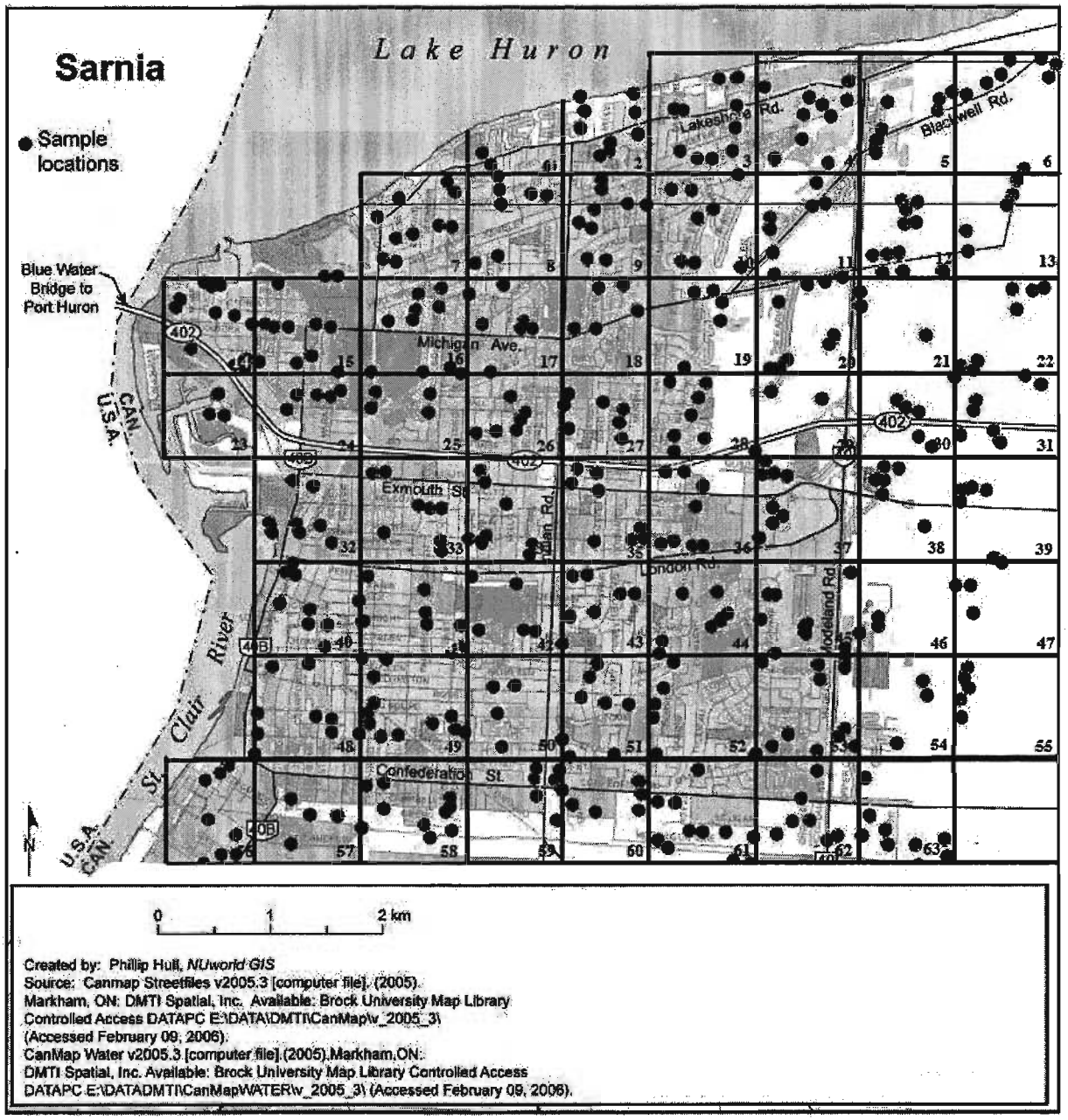


Figure 3.1. The location of the 63 grid squares and the locations of the trees sampled in the main survey.

The UTM coordinates were obtained using a Garmin Rino 530 WAAS enabled 12 channel handheld unit with a maximum resolution of ± 5 m. The UTM coordinates,

the diameter at chest height and the lichen survey data were noted on a preformatted data collection sheet. Five lichen community indicators were examined: lichen species richness, dominant lichen species, second dominant lichen species, percent lichen cover and IAP. The EMAN lichen suite for mixed-hardwood forest lichens (Brodo and Craig, 2001) was used for lichen identification and nomenclature. This suite was used in order to avoid misidentifying lichens since a few species appear similar. Misidentification was also avoided by examination with a hand lens since the species that appear similar to those in Sarnia have differentiating features (e.g. different textures, fruiting structures). If further uncertainty still existed, the lichen in question was to be collected for further identification. The lichen data were derived from visual surveys at a height of 0.5 to 1.5 m on the trunk of each tree.

The maximum number of lichen species present (lichen species richness) was recorded for each tree. The two lichen species that covered the greatest portion of the surveyed part of the tree were identified as the dominant and second dominant lichen species. This was done by visually assessing the surveyed portion of each tree. The percentage of lichen cover on a tree was determined by visual estimation and by the line-intercept method. Visual estimates were done by two workers for each tree. These visual estimates of percentage lichen cover are assumed to be nominally accurate to ± 10 %. The line intercept method sums the widths of all lichen thalli that made total contact with a painter's tape that was wrapped around the tree at 1.5 m in height. This value is termed the 'line intercept' and was found to have a nominal accuracy of ± 5 %.

Percent Lichen Cover = (Circumference – Total Line Intercept)/100

Equation 3.1 Percent Lichen Cover Equation. Tree circumference and lichen cover are in centimetres.

The values from the line intercept method were compared with those from the visual method in order to assess the accuracy of the visual method. The visual method was found to be comparable to the line intercept method, so the visual method was used in the statistical analysis due to the easy field application for other studies. The circumference of the trunk in centimetres was measured at chest height with a fiberglass tree diameter tape (nominal accuracy of ± 2 %) and an IAP value was calculated for each plot using the eight tree survey data and Equation 2.1. In order to identify slight differences in IAP values between the sites, seven IAP classes will be used initially. The number of classes were then decreased to three in order to identify broad trends across the entire study area.

3.2 Habitat and Land Use Variables

Habitat variables were documented for each tree. The variables are tree diameter at chest height, distance to the nearest road, distance to a lake or river, canopy cover and the Index of Human Impact (IHI) (Equation 3.2). The distance of each tree to the nearest road was calculated using a measuring tape extending from the base of the trunk to the edge of the street. Light levels at chest height (canopy cover) were measured as Lux using a handheld light meter with a diffusion bulb (Extech instruments Model 401025, resolution 1 Lux, nominal accuracy of ± 5 % and 2 digits). One reading was taken with the probe held at chest height about 2 cm from the trunk where there was the greatest lichen cover. Soon after, a second reading was

taken in direct sun about 3 m outside the canopy. A ratio of the amount of light recorded under the canopy to the amount of light recorded outside the canopy was then generated based on the two readings. All measurements were recorded under clear skies.

A qualitative estimate of the artificiality of each site was subjectively classified through the use of the IHI in the field at the same time as the trees were surveyed. Four variables necessary for the calculation of the IHI (Gombert *et al.*, 2004) were assessed: Urbanization, Traffic Volume, Local Developments and Exposure. Weights ranging from 0 up to 4 were assigned to the different variables to provide a quantitative expression of the human impact of the site (Table 3.1).

The Index of Human Impact = U (T+D+E)

U = Urbanization, T = Traffic Volume, D = Local Developments, E = Exposure of Trees

Equation 3.2 The Index of Human Impact (Gombert *et al.*, 2004)

Table 3.1 The Index of Human Impact (IHI). This index is used to assess the extent of human activity at a site. Modified after Gombert *et al.*, (2004) to include a third weighted value for the Urbanization variable.

U (Urbanization)	T (Traffic Volume)	D (Local Developments)	E (Exposure of Trees)
0 rural	0 no roads	0 green space	0 isolated
1 suburban	1 two-lane road	1 building smaller than average two storey house	1 in groups
2 urban	2 parking lot	2 building larger than two storey house	
3 industrial	3 four lane road		
	4 highway		

Urbanization is a measure of the density of buildings, population and infrastructure. It has four classes ranging from zero or rural to three, industrial. Rural was defined as a location that was either used for agricultural purposes or left in its

natural state with no buildings. A location was considered suburban if it was in a residential area in which most of the buildings were detached single-family houses. An urban location was a densely populated area with a high concentration of buildings and infrastructure. Industrial locations were areas in which the main buildings and infrastructure were used primarily for industrial purposes. The industrial class was not a part of the original equation used by Gombert *et al.* (2004). This was added due to the large petrochemical industry in Sarnia.

Traffic volume was used to assess the frequency and magnitude of vehicle traffic and to qualitatively estimate the amount of exposure of the site to traffic emissions. It has five classes which are based on the number of lanes within 10 m of the tree being surveyed.

Local Developments refer to any man-made structure that could block sunlight or air flow. This includes buildings and sheds. It has three classes. A location with no structures within 10 m was classified as a green space. A classification of one was assigned if there was a building smaller than a two storey house, such as a shed within 10 m. If there was a building larger than a two-storey house within 10 m, then a classification of two was assigned.

The final variable was Exposure. One of the requirements for a tree being surveyed was that it was open grown. Therefore, E was zero for all sites. Table 3.1 lists these categorical data. Equation 3.1 is the original equation presented by Gombert *et al.* (2004). Where a different IHI value was found for any of the eight trees in a plot, the average of the eight trees was used as the IHI value for the plot.

The distance from the nearest water body is the straight line distance from the centre of the grid to the lake and the river. This was measured using the Google Earth Pro program by Google and is accurate to ± 5 m. This measure assumes much about humidity gradients and simplistically assumes that buildings and land use have little influence on humidity levels. As such, it is a tentative characterization of humidity differences and may have weak explanatory power.

3.3 Substrate pH

In September of 2006, bark samples were taken from mature, open grown *Acer sacchrum* trees with circumferences of >64 cm in Sarnia, Hamilton and Windsor. Bark samples were taken from 60 trees in Sarnia. Fewer samples were collected in Hamilton (N = 54) and Windsor (N = 31) as suitable trees were not available for bark collection. In order to efficiently characterize the impact of variability of bark pH on lichen communities caused by contamination, thirty trees were selected for bark sampling within 10 m of a known source of contamination (*e.g.*, a busy, large parking lot or a four lane road). These samples were classified as being from 'dirty sites'. An additional thirty trees were selected with no known source of contamination within 10 m in areas such as parks and green spaces. These samples were classified as being from 'clean sites'. Bark samples were approximately 3 cm^2 with a depth of no greater than 4 mm in order to avoid the natural variation between the outer and inner bark. Sample weight was approximately 1.5 g. Samples were collected from the area adjacent to the greatest lichen cover at a height between 0.5 and 1.5 m using a chisel. Areas with nutrient streaks or signs of damage or insect infestation were avoided. Clean bark was preferred over lichen-covered bark as some

lichens secrete acidic solutions that may naturally alter the bark pH (Farmer, Bates and Bell, 1990; Van Herk, 2007). Once removed, the samples were stored individually in plastic bags with the site number and species richness values recorded on them and were air dried at 20 °C for three weeks. When thoroughly dried, the bark pieces were cleaned of any detritus using a wooden toothpick and then manually broken into pieces approximately a centimetre in diameter.

The bark pieces were individually ground to a uniform powder using a burr coffee grinder. Once ground, the sample was then relocated to a labelled Petri dish. The samples were each weighed to the nearest 0.25 g using a Mettler P1200 digital balance. The grinder was thoroughly cleaned using compressed air between each sample. Three amounts of 0.5 g from each sample were mixed thoroughly by hand for one minute with 15 mL deionised water in beakers covered with parafilm. The samples were put in a Mettler C200 centrifuge for an hour to separate the ground bark from the liquid to allow for spectrophotometric analysis and then left to settle over night. The following day the mixtures were centrifuged again for 15 minutes, allowed to settle then re-shaken for five minutes by hand. The samples were then filtered using Whatman No. 4 filter paper and deionised water. The pH was measured using a temperature compensated Corning pH meter. Pre-mixed reference solutions from Hach were used in order to calibrate the Corning pH meter. The pH levels of the three solutions were 4, 7 and 10. The probe was rinsed with deionized water and wiped clean after every measurement. It was recalibrated after every third reading and checked against a solution with a known pH. The pH probe was left in the solution for fifteen seconds in order to ensure accuracy. The meter was then calibrated to

reflect the known pH of the calibration solutions. The probe was allowed to remain in the solution until it had stabilized for fifteen seconds. After each reading the probe was rinsed with deionized water.

3.4 Data Processing

All lichen species and habitat data from the 64 sites were entered into a Microsoft Office Excel ™ ver. 12 database and were analyzed using Microsoft Office Excel™ ver. 12 and Minitab Statistical Software™ ver. 15. Descriptive statistics were generated and simple correlation tests were performed on each data set. The descriptive statistics, including mean, maximum, minimum and the standard deviation; and the correlation coefficients were calculated for each variable. Lichen community classification was done using the method of Kershaw (1975).

3.5 Geostatistical Analysis

Controlled access digital map files for the City of Sarnia were provided to Dr. D. McCarthy by the Brock University Map Library. The files were then upload into Geographic Information System software (ArcGIS's Geostatistical Analyst™ extension, 2007) which was used by Philip Hull under close supervision to create a continuous surface that was based on the 456 trees surveyed in the study area. Exploratory Spatial Data Analysis (ESDA) tools (ArcGIS™, 2007) were used to examine the data and a kriging technique was used to interpolate values. In the early stages of kriging, histograms were created using the Geostatistical Analyst Extension™ (2007) and the data array was subdivided into classes that were optimized by the software. Ordinary kriging and default parameters were used. In order to produce a predicted surface map, the Kriging method was set to Ordinary,

Prediction Map without transformations or the removal of trends. Ordinary kriging creates an estimate for the mean value. In order to confine the predicted surface map to the study area, the properties of the layers were changed using the Clip to Shape feature. The default setting of Filled Contours was selected in order to display the interpolated surface.

4.0 RESULTS

4.1 Preliminary Survey

The initial survey examined two hundred mature, open grown *Acer* trees with diameters greater than 76 cm at chest height. Trees meeting the criteria for selection could not be found at twelve sites (sites 1, 21, 22, 28, 29, 36, 37, 38, 46, 53, 54 and 63 in Figure 3.1) and only three per site were found at sites twelve and forty-three (Site coordinates are listed in Appendix IV). Four species of *Acer* were found in the study area; *Acer platanoides* L., *Acer rubrum* L. var. *trilobum* Torr. & A. Gray ex K. Koch, *Acer saccharinum* L. *and Acer saccharum* Marsh.

Lichen thalli were found to be the most abundant on *Acer saccharum* (Table 4.1). Nine lichen species were identified in the study area; *Physcia millegrana* Degel, *Candelaria concolor* (Dicks) B. Stein, *Physcia aipolia* (Ehrh ex Humb.) Furnrohr, *Phaeophyscia chloantha* (Ach.) Moberg, *Candelariella efflorescens* (Harris & Buck), *Parmelia sulcata* Tyl., *Physcia adscendens* Oliv, *Xanthoria fallax* (*sensu stricto*) and *Parmelia caperata* (L.) Ach. The majority of the lichen thalli were small (*e.g.*, less than 5 mm in diameter).

Table 4.1. The four most common species of *Acer* with diameters at chest height greater than 76 cm found in Sarnia during the initial survey and their maximum, minimum and mean lichen species richness (Nomenclature according to USDA, 2009).

Species	Total # Surveyed	Maximum	Minimum	Mean
Acer sacchrum	*53*	9	0	5.5
Acer platanoides	*57*	8	0	5
Acer rubrum	*41*	5	0	3
Acer saccharinum	*45*	4	0	3

4.2 Main Survey

Even with the decrease in the required trunk circumference for the selection of trees, 6 sites (most of which were located in the rural southeastern sector of the city) lacked a total of 8 trees which met the criteria. This resulted in a total of 458 trees in this survey. While due to a lack of trees, the decrease in the required circumference allowed for at least partial surveying at these locations as at least several trees were identified at these sites. Trees that had been surveyed in the initial survey were omitted from the main survey.

4.2.1 Lichen Community Composition and Frequency

Eleven epiphytic lichen species were identified on the 458 trees surveyed in the main survey. Eighteen trees had no identifiable lichen cover. *Physconia detersa Nyl. Poelt.* (at sites 3, 5, 6, 12, 13, 29, 31 in Figure 3.1) and *Parmelia subaurifera Nyl.* (at sites 3, 7, 9, 13, 27 and 42 in Figure 3.1) were identified in the main survey which were not found in the initial survey. The species identified in this study have been found in other studies (e.g. LeBlanc & DeSloover, 1970; van Herk, 2001 and Washburn, 2005) which were identified as having similar contamination levels. The number of species identified at each site ranged from zero to nine lichen species. A mean of 3.3 lichen species for all of the trees surveyed was found as seen in Figure 4.1.

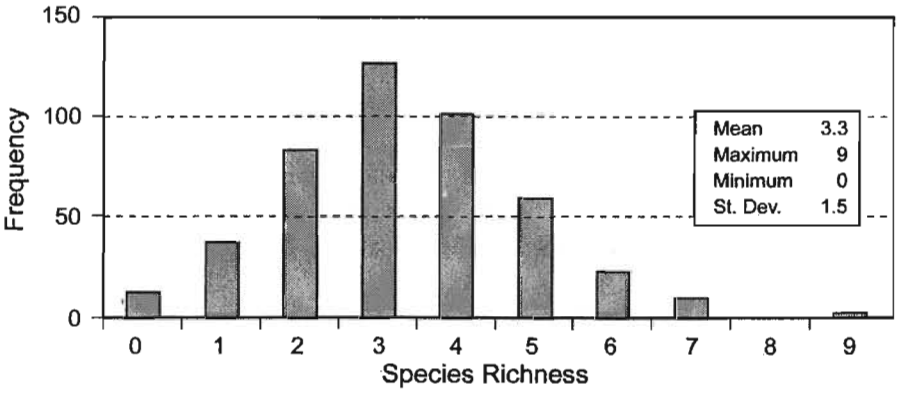


Figure 4.1 Histogram showing lichen species richness values from the main survey of Sarnia of 458 trees).

GIS was used to produce a map displaying the spatial distribution of lichen species richness for Sarnia (Figure 4.2). Higher lichen species richness values (between six and nine lichens) were found in the northwest; lower lichen species richness (between zero and two lichens) was found in the east.

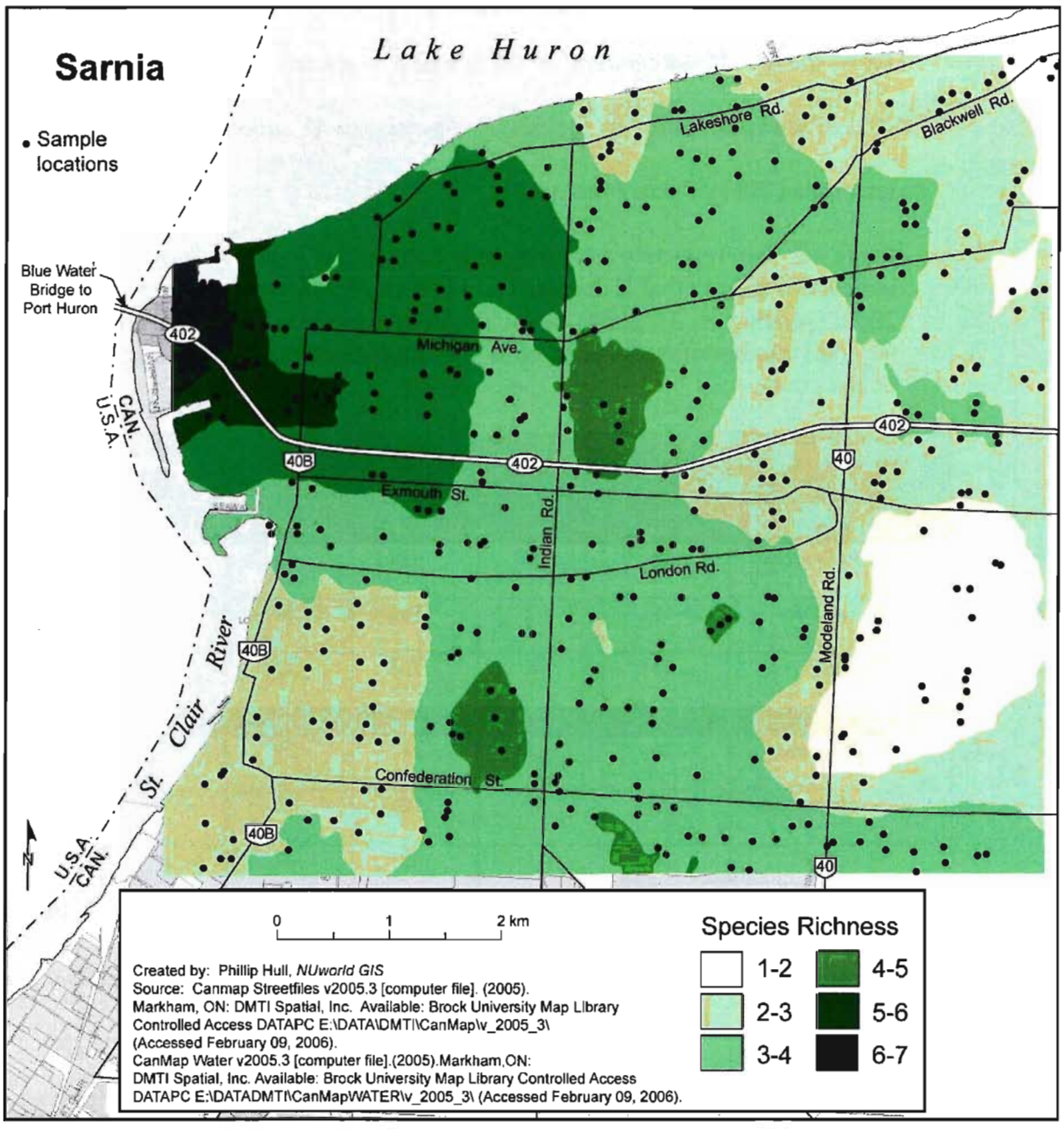


Figure 4.2 Map showing maximum lichen species richness and the location 458 trees surveyed.

Table 4.2 displays the frequency and rarity of the 11 lichen species identified during the main survey. Three lichen species, *P. millegrana, C. concolor* and *P. aipolia* were identified at over 70% of the 63 sites surveyed in the second study. More

than 40% of the sites were found to have *P. chloantha, C. efflorescens* and *P. sulcata.* Three species, *P. adscendens, X. fallax* and *F. caperata* were identified at more than 20% of the sites while two species; *P. detersa* and *M. subaurifera* were found at fewer than 20% of sites (Table 4.2).

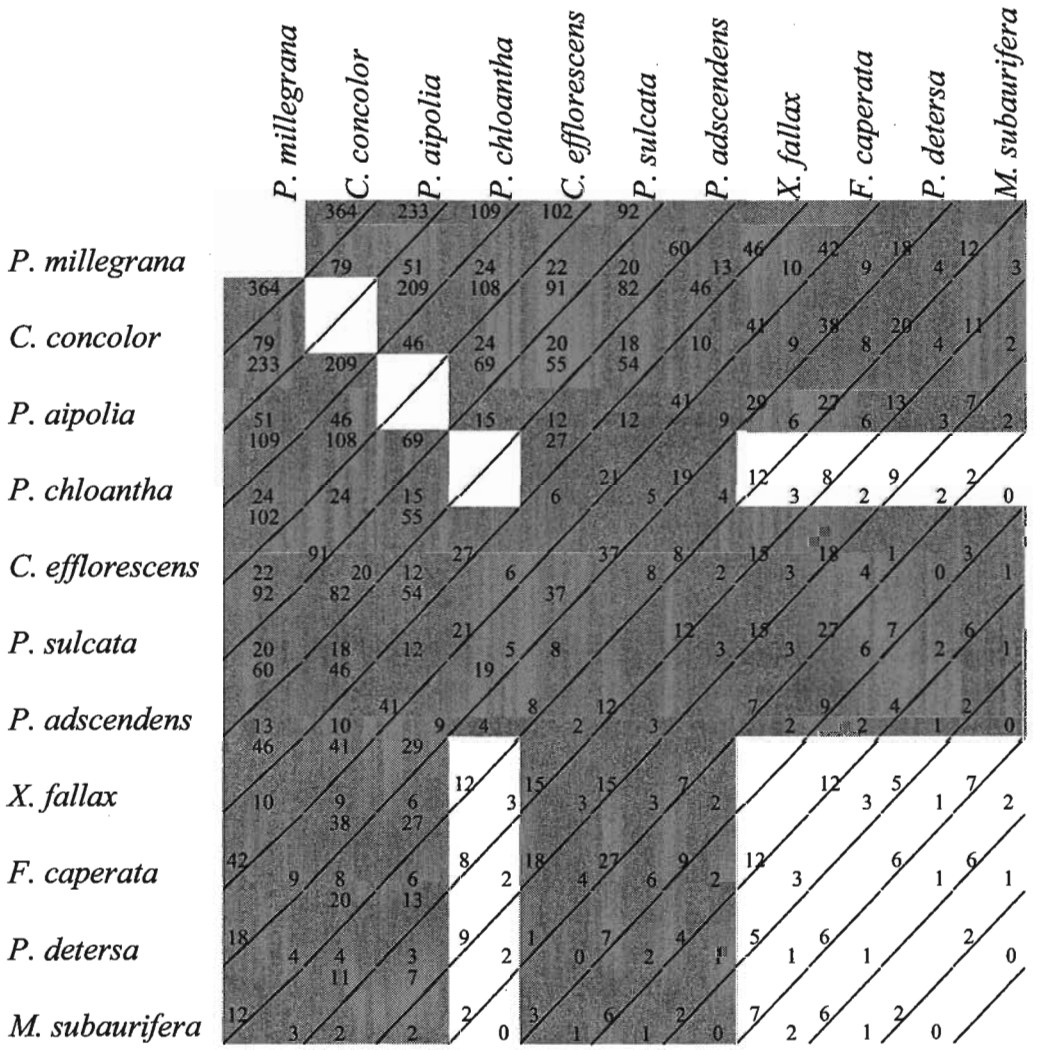
Table 4.2 The frequency and rarity of lichen species found in Sarnia. The asterisk indicates a species that is accepted as a known nitrophyte in the literature (McCune, 2000; Gombert et. Al, 2004). No acidophytes were identified. Total number of sites is 63, total number of trees surveyed is 458. Eighteen trees did not have identifiable lichen cover.

Species	# of Sites	% of all Sites	# of Trees	% of Trees	% of all Lichens	Rarity
						Common
**P. millegrana*	63	100	440	96	29	Found on over 50% of trees.
**C. concolor*	58	92	365	80	24	Over 10% of all lichens.
**P. aipolia*	48	76	236	52	16	Found at over 70% of sites.
						Medium
P. chloantha	26	41	111	24	7	Found on over 20% of trees.
**C. efflorescens*	26	41	102	22	7	Over 5% of all lichens.
**P. sulcata*	26	41	92	20	6	Found at over 40% of sites.
						Uncommon
**P. adscendens*	17	27	60	13	4	Found on over 5% of trees.
X. fallax	16	25	46	10	3	Over 2% of all lichens.
P. caperata	14	22	42	9	3	Found at over 20% of sites.
						Rare
P. detersa	7	11	20	4	1	Found on less than 5% of trees.
M. subaurifera	6	10	12	3	0.01	Less than 1% of all lichens. Found at less than 20% of sites.

Lichen communities were examined to determine whether several lichen species were usually found together, suggesting similar habitat, substrate or air quality. The

beginning step of this was to examine the frequency in which two species were found together in Table 4.3.

Table 4.3 The frequency of occurrence for two lichen species on the same tree. Superscript numbers indicate the number of trees at which both species were present. Subscript numbers indicate the percentage of all trees (n = 458) that had both species. Shaded cells represent at least one species being listed as a nitrophyte.



Community groups were identified as being species that were found together on 50% of the trees on which one species was identified. Only three lichen species, *P. millegrana, C. concolor* and *P. aipolia*, showed any evidence of community

grouping with frequencies of greater than 51% (Table 4.3). All three species are nitrophytes. As shown in Table 4.2, *P. millegrana* was the species with the strongest relationships with other species and was identified at more sites than the other two common species so it was listed at the top of Figure 4.3.

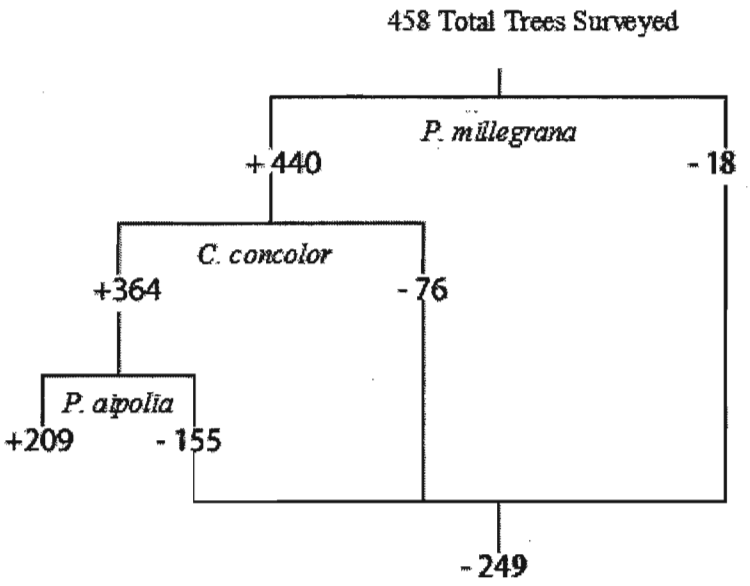


Figure 4.3 Classification of the "common" nitrophytic lichen community found in Sarnia. + indicates the number of trees surveyed with all the species above present, while – indicates the number of trees surveyed without all the species above.

The number of trees upon which *P. millegrana* was identified (n = 440) was subtracted from the total number of trees surveyed (n = 458). From this it was determined that eighteen trees did not have *P. millegrana* or any other lichen present at an identifiable size. Step two was completed by subtracting the number of trees with *C. concolor* (n = 364), the second most common species, from the total number of trees with *P. millegrana* (n = 440). This determined that 76 trees did not have both *P. millegrana* and *C. concolor*. The third step examined the relationship between *P.*

aipolia and the other two species. Of the 364 trees that had both *P. millegrana* and *C. concolor*, 209 trees also had *P. aipolia*. This led to 155 trees with only *P. millegrana* and *C. concolor* with no *P. aipolia*. The trees without each species were then added to determine the number of trees that did not have this community grouping out of the total number of trees surveyed which were 249. Of all the trees surveyed, 46% were found to have this community grouping.

All lichen species classified as 'common' are species that have been identified as nitrophytes in the literature (see Table 4.2); a total of six of the eleven species identified in Sarnia are known nitrophytes. Neither of the species classified as 'rare', *P. detersa* and *M. subaurifera,* are known nitrophytes. The other five species are suspected nitrophytes or are neutrophytes as they are generally found in locations with poor air quality and high bark pH values. No acidophytes where identified.
The percentage lichen cover on all trees surveyed (n = 458) was examined for spatial patterns. Statistical testing of all the trees surveyed (n = 458) was done in order to determine whether the differences between the values generated by the two different methods was statistically significant. A two-tailed F-test at a 95% confidence level confirmed that the variance of the two sets of data was the same (F= 1.08). A Z-test at a 95% confidence level was also run and showed that the difference between the two means was small (Z = 0.1401). Since the difference in values was not statistically significant between the two methods, the data from the visual method will be used in statistical analysis with other variables. Lichen percentage cover follows a similar spatial distribution to the lichen species richness map. Trees with the greatest percent lichen cover were generally located in the northwest sector of the city.

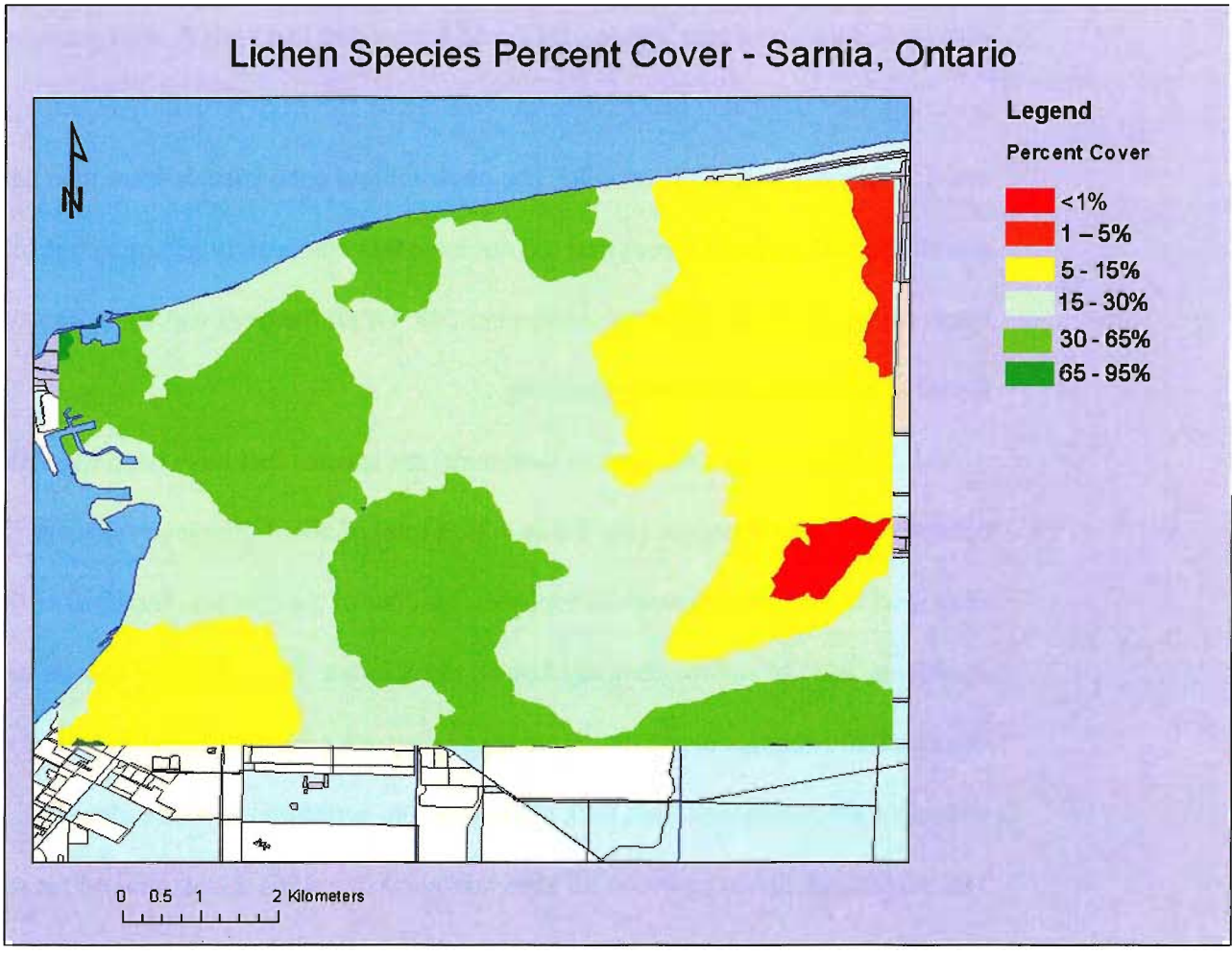


Figure 4.4. Percent lichen cover for all trees using the visual method (n= 458). Classes were based on the natural divisions of the data. Classes of less than 5% were added since lichen thalli may have been present but may be too small to be visually identified.

Figure 4.4 shows the spatial distribution of percent lichen cover categories within the study area. Lichen percentage cover ranged between 15 and 65% through the city center to the southern portion of the study area. An area of very low percent cover, 0 – 15% exists to the east of the city where the land use is predominately rural. A second area of low percent cover, 5 – 15% is located in the south-west sector of the study area, close to a chemical plant.

The 19 IAP values that were calculated for the study area were divided first into seven categories to produce an in-depth overview of IAP value spatial distribution. The IAP values were later divided into three categories to give a broad overview of the spatial distribution. Figure 4.5 displays the spatial distribution of IAP values in seven categories in the study area. A small cluster of sites with values between zero and six were found near the southwestern sector of the study area near industrial plants. IAP values greater than 15 were identified in the northwestern corner of the study area near the Bluewater Bridge which experiences heavy cross border traffic. Since IAP values are directly related to species richness values, Figure 4.5 displays trends similar to that of Figure 4.2. IAP values tend to decrease with increasing distance to the east of the study area where land use becomes rural rather than urban.

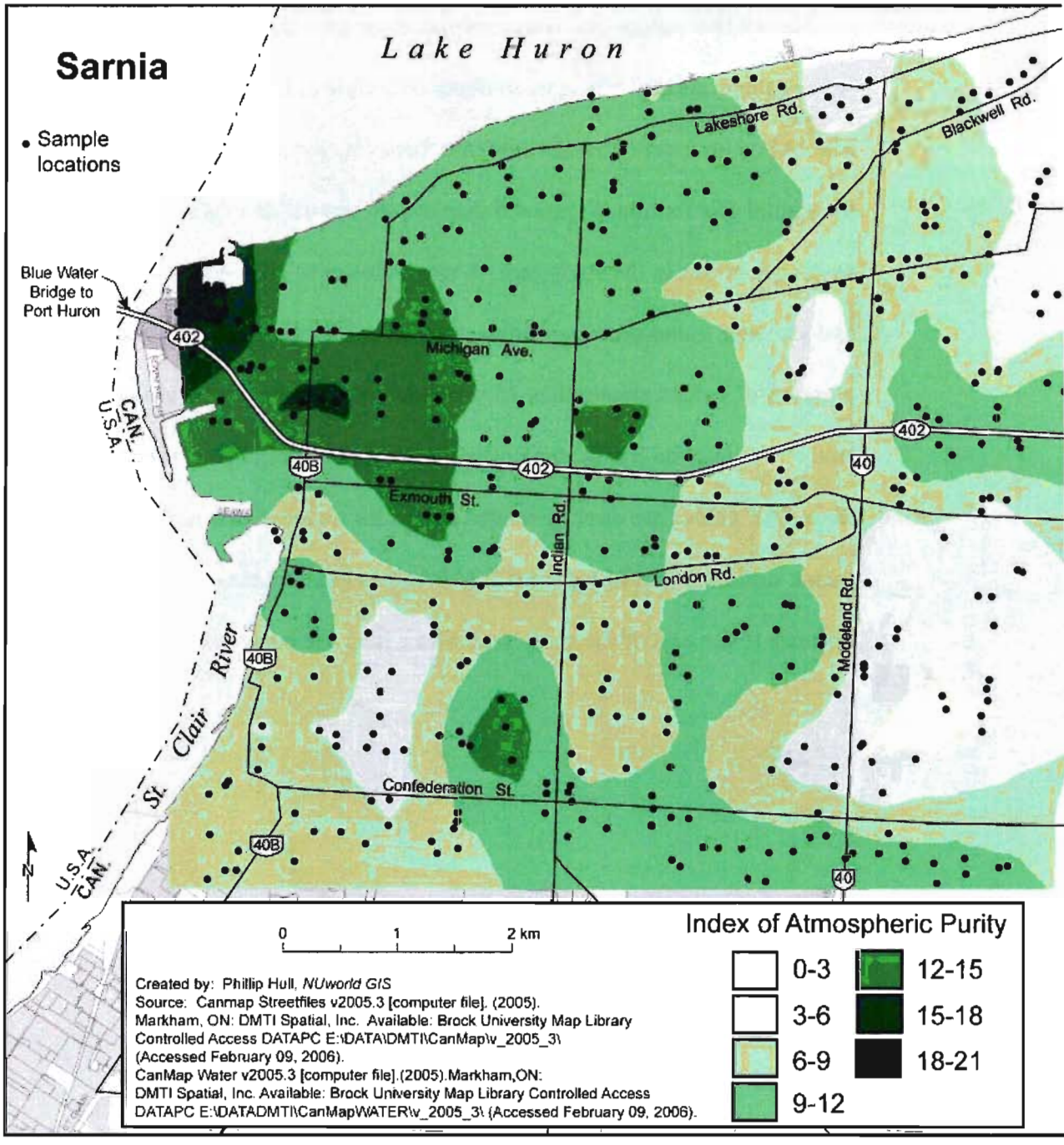


Figure 4.5 The spatial distribution of IAP values (n = 458).

The IAP values were divided into three categories based on natural breaks in the data in order to provide a broad view of the IAP zones of the study area, as shown in Figure 4.6. Values ranged from 2.61 to 19.68. The average IAP value was 8.77 and the standard deviation was 3.40.

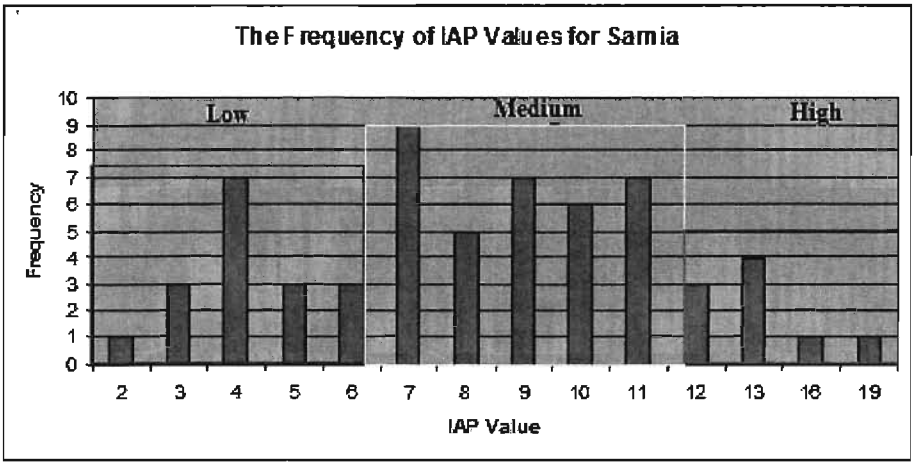


Figure 4.6 The frequency of IAP values for the 63 1km^2 sites surveyed in Sarnia.

Figure 4.7 displays the spatial distribution of the three IAP categories in the study area. Sites with IAP values between 0 and 6 were classified as having low IAP values in comparison to the values of the rest of the surveyed area. Sites with IAP values ranging from 7 to 12 were classified as medium. IAP values greater than 12 were classified as being high. The IAP categories can also be linked to nitrophytic species. A site was considered to be dominated by known nitrophytic species (as listed in Table 4.2) if nitrophytes outnumbered non-nitrophytic species. Trees surveyed in sites with low IAP values were dominated by nitrophytic species. Sites where IAP values were low (69% of the trees surveyed) were dominated by nitrophytes. Only 8% of the trees surveyed in the sites that were classified as having fair ambient air quality were dominated by nitrophytic lichen species.

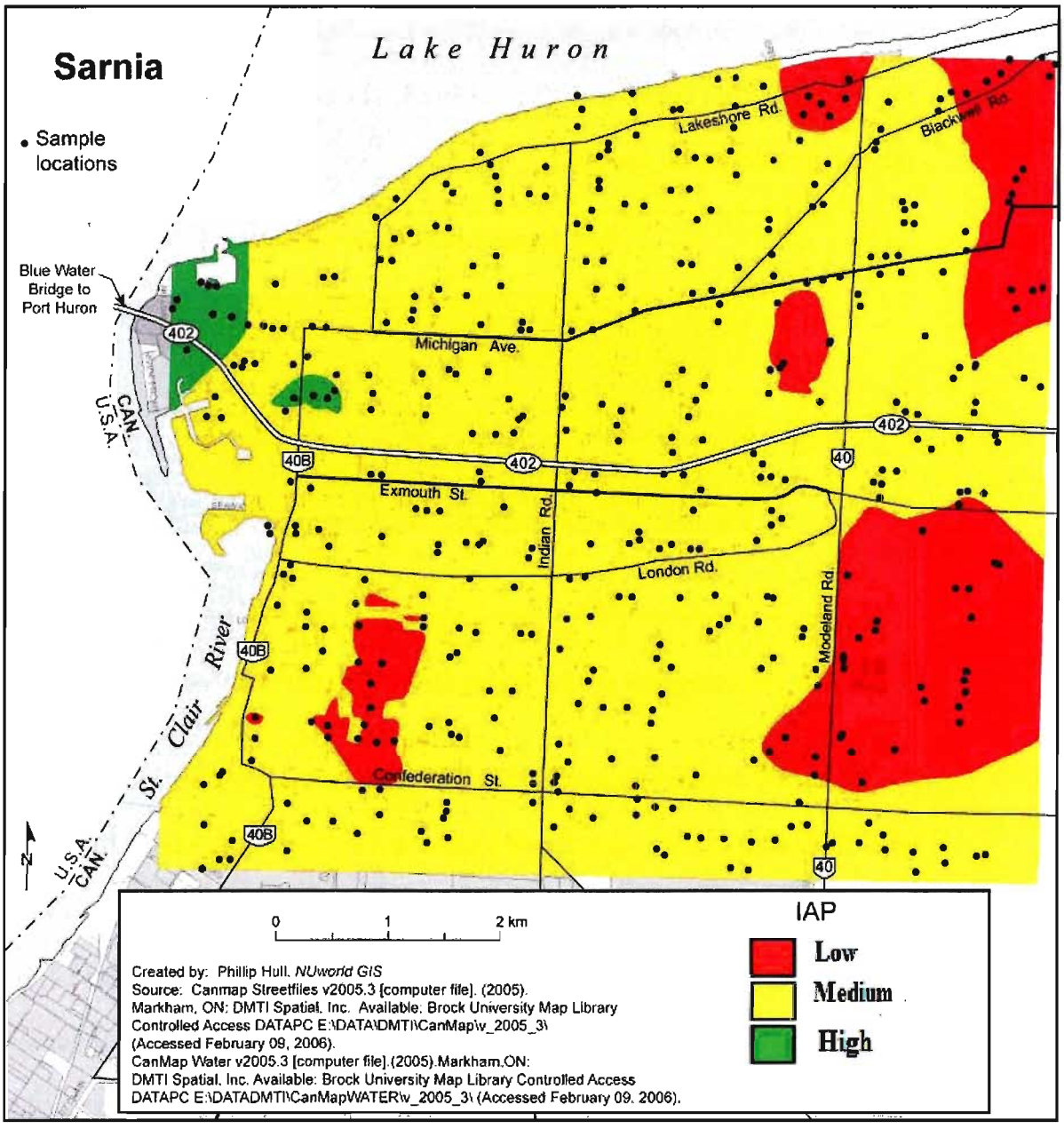


Figure 4.7 The spatial distribution of the three IAP categories, low, medium and high.

Sites with low IAP values account for approximately 24% (rounded) of the sixty-three 1 km^2 sites in this survey. Most of these sites are located close to rural areas to the west of the city center.

Medium IAP values were found in approximately 74% (rounded) of the sites and tended to be located in the northwest sector of the study area and extended down to the city centre. Sites with medium IAP values border Confederation Street in the south of the study area (Figure 4.7). Only three sites in the study area had high IAP values of 12 or greater. These sites made up only approximately 5% (rounded) of all sixty-three sites studied. All three sites were located in the northwestern sector of the study area in an area known for recreational activities combined with green spaces.

4.2.2 Habitat and Land Use Variables

Attempts to statistically correlate the lichen community variables against habitat and land use variables returned several very weak correlations ranging from -0.52 to 0.52 (Appendix III). The IAP and IHI values for all 63 sites surveyed were examined. No strong relationship ($r^2 = 0.32$) was found between the two (Appendix III). A polynomial approach explained a low variance of 19% which was higher than the linear regression which accounted for only 4%.

4.2.3 Bark pH and Lichen Species Richness

Sixty trees from Sarnia were tested for bark pH. Samples from 31 trees in Windsor and 54 in Hamilton were also collected and tested. Variations in the number of tests are due to the number of suitable trees and the amount of bark available for sampling. The lichen species richness value of each tree from which samples were collected was recorded. Table 4.4 displays the descriptive statistics for the bark pH and lichen species richness. The pH values ranged from 4.5 to 6.1. The highest pH value (6.1) was identified near the city center of Sarnia. Table 4.5 displays the

differences in the descriptive statistics between the 'clean' and 'dirty' sites in Sarnia, Hamilton and Windsor.

Table 4.4. Descriptive statistics for the bark pH and lichen species richness from Sarnia, Windsor and Hamilton.

	Sarnia		Windsor		Hamilton	
	pH	Richness	pH	Richness	pH	Richness
Mean	5.5	4.5	5.6	3.6	5.8	3.2
Maximum	6.1	9	6.7	7	6.6	5
Minimum	4.5	0	5.1	1	5	0
N	60	60	31	31	54	54

Table 4.5. Summary of bark pH data and lichen species richness from clean and dirty sites in Sarnia, Hamilton and Windsor.

	Sarnia		**Windsor**		**Hamilton**	
	Clean	Dirty	Clean	Dirty	Clean	Dirty
pH						
Mean	5.6	5.4	5.6	5.6	5.8	5.9
Maximum	6.1	6	6.1	6.7	6.4	6.6
Minimum	5.1	4.5	5.1	5.2	5	5
Richness						
Mean	2.9	3.8	4.7	2.7	3.2	3.5
Maximum	6	7	5	4	7	5
Minimum	1	1	3	1	2	0
N	30	30	16	15	27	27

Relationships were discovered between lichen species richness and pH when the two classifications of sites were analyzed separately. A positive relationship between these two variables (Figure 4.6) was identified for dirty sites. A correlation coefficient of 0.8 was found for pH and species richness at dirty sites (Figure 4.8). A negative relationship was found between lichen species richness and pH for clean sites (Figure 4.9). A correlation coefficient of -0.7 was calculated for species richness and pH at clean sites.

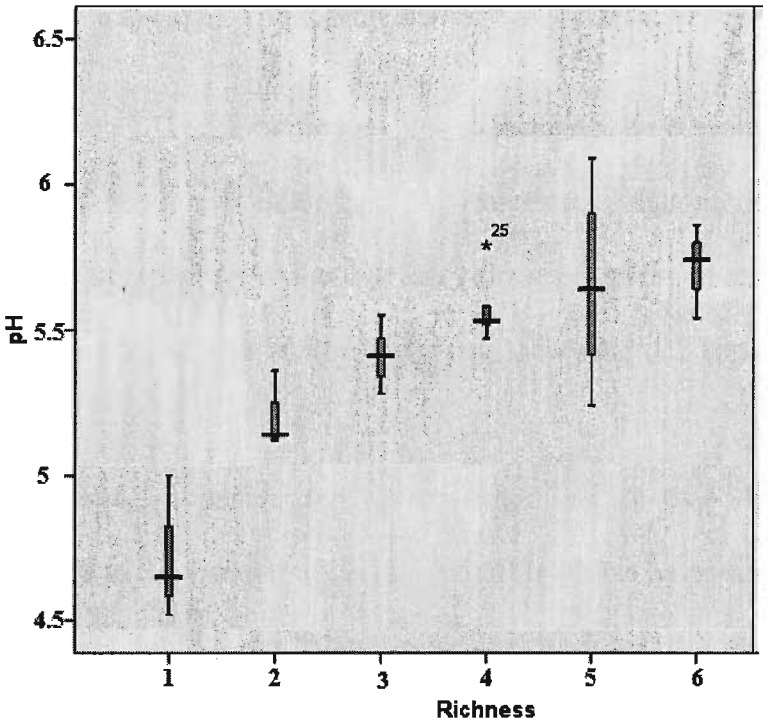


Figure 4.8 The lichen species richness at 'dirty' sites in Sarnia. The middle horizontal lines on the bars display the mean while the top and bottom horizontal lines represent the maximum and minimum. The boxes show the values that fall within the 25th and 75th percentiles while the vertical lines represent the expected range of values. An outlier is denoted by *25.

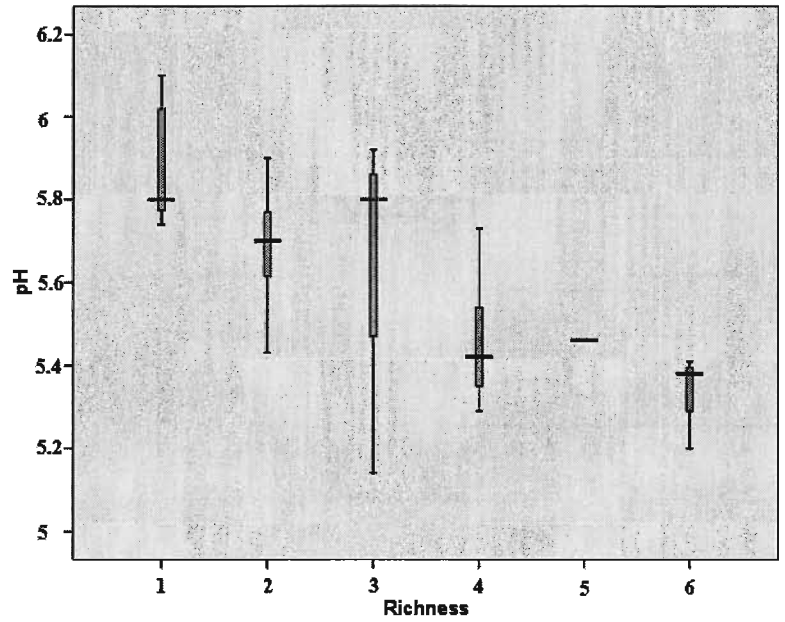


Figure 4.9 The lichen species richness at 'clean' sites in Sarnia. The middle horizontal lines on the bars display the mean while the top and bottom horizontal lines represent the maximum and minimum. The boxes show the values that fall

within the 25th and 75th percentiles while the vertical lines represent the expected range of values.

When the bark pH values were examined in conjunction with the IAP categories a relationship not displayed through linear regression or correlation emerged. Sites that were suspected to have poor ambient air quality due to low IAP values were found to have an average pH value of 5.9. Sites with higher IAP values were found to have an average pH of 5.5.

As displayed in Figure 4.10, the five lichen species most commonly found in Sarnia at the sixty sites surveyed exhibit different ranges for the bark pH at the trees surveyed. No difference was found for *P. millegrana* and *P. chloantha* for pH. These two lichen species were found to exist on trees with the greatest range in pH values examined in this study. *Candelaria concolor* favoured a lower pH range than the other species. *Xanthoria fallax* had a limited range for pH when compared to the other lichen species (Figure 4.10).

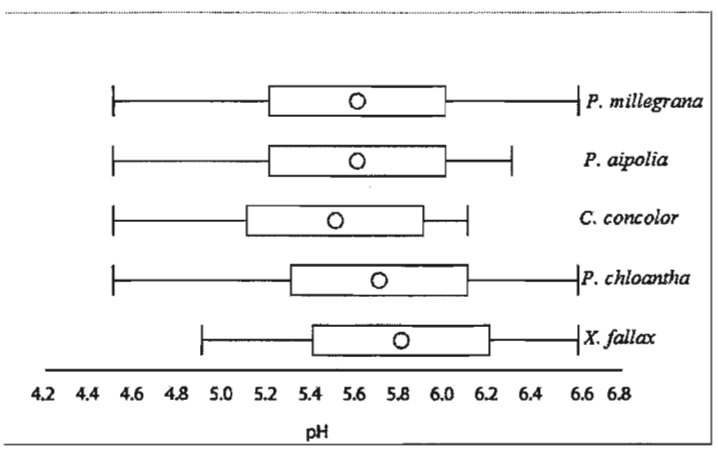


Figure 4.10 Descriptive statistics shown in box and whiskers plots for the five most frequently occurring lichen species in Sarnia and substrate pH. The circles denote the mean. The vertical lines on the left represent the minimum, while the lines on the right represent the maximum. The boxes show the values that fall within one standard deviation of the mean.

The nitrophytic lichen community of *P. millegrana, C. concolor* and *P. aipolia* were found on bark that has a mean pH of 5.5. All three species inhabit trees with a mean pH value of 5.5. Each of the five most commonly identified lichen species in the three cities appeared to inhabit trees with different pH. The range of values of pH that seem to be tolerated by the different species were divided into three categories, high, medium and low based on the pH level in comparison to the levels found with the other lichen species (Table 4.7). *Xanthoria fallax* was found to be the most sensitive species to low pH levels of the five most common species identified in Sarnia.

Table 4.7. Characterization of lichen species and their possible tolerance to a large range of pH levels based on those of the other lichen species examined.

Species	pH Range	Classification
X. fallax	4.9 – 6.6	low
P. chloantha	4.5 – 6.6	high
C. concolor	4.5 – 6.1	low
P. aipolia	4.5 – 6.3	moderate
P. millegrana	4.5 – 6.6	high

5.0 DISCUSSION

This chapter discusses the results of the survey beginning with lichen communities, spatial trends and habitat variables and concluding with a discussion of substrate properties and individual lichen species.

5.1 Lichen Communities and Frequency

Less than 12 lichen species were found at 92% of trees in the study area. The average lichen species richness was 3.3. This is more consistent with the linear relationship observed in Figure 1.1. Only 8 % of all the sixty-three sites surveyed in Sarnia displayed lichen species richness values of less than twelve. The spatial distribution of lichen species richness, percent lichen cover and IAP values were very similar. Most of the study area had low IAP values (between 0 – 6) and low to moderate percent lichen cover values of < 30%.

Sarnia's arboreal lichen community was found to be almost evenly split between known nitrophytes (55 % of those found) and neutrophytes or suspected nitrophytes (45 %) with no known acidophytes being indentified (Gombert *et al.*, 2003; Sparrius, 2006). Only two lichen species in the EMAN suite, *Lobaria pulmonaria* (L.) Hoffman and *Usnea diplotypus* Vain, are cyanolichens. These lichen species, which contain cyanobacteria that fixed atmospheric nitrogen and are considered to be very sensitive to air quality, were also not found in Sarnia. The most common lichen community in Sarnia is one composed of the known nitrophytes: *P. millegrana, C. concolor* and *P. aipolia.* These three nitrophytic species were found with the greatest frequency and percent cover. *Physcia millegrana* was the dominant species on 86%

of the trees. *Candelaria concolor* was the second dominant on 56 % of all trees surveyed while *P. aipolia* was the second dominant species on 20 %.

5.2 Habitat and Land Use

A spatial relationship was found between lichen species richness, IAP and percentage cover and proximity to the Bluewater Bridge and Highway 402. Sites less than 1 km from the bridge and highway displayed greater IAP, lichen species richness and percent cover values than other sites. Lichen species richness values were uniform throughout the city centre, possibly due to diffuse eutrophication, with the exception of the bridge and highway noted above and a slight decline in the southwestern sector of the city close to heavy industry. The presence of regional emissions from long distance transport may hinder attempts to isolate local trends by creating a high background level of diffused contaminations.

5.3 Bark pH

As the previous discussion suggests, lichens do not provide a clear indication of the processes responsible for their spatial distribution. Lichens can respond to a variety of stressors, some of which may be unknown. Since the time required for lichen colonization and growth of the species in the EMAN suite is unknown, lichen communities may not necessarily be reflective of the present ambient air quality conditions. Substrate properties such as pH (van Herk, 2007) evolve over time. Consequently, air quality may improve but the bark may still remain free from lichen cover because it still contains contaminants deposited in the past. This study found that tree bark can be used to establish a link between lichen species richness and pH.

There is no generally accepted method for tree bark collection and analysis, and

since studies are limited to tree species common to their study areas, little background information exists on the normal chemical composition of maple bark. Thus, it is difficult to compare data from different studies. The method used in this study was modified after van Herk (2007). That study found that an increase in bark pH and a low sensitivity of nitrophytic species to SO_2 appeared to be responsible for a large increase in the number of nitrophytic species and a lack of acidophytic species.

The bark samples collected from the sixty trees sampled in Sarnia had a mean pH of 5.5 which was lower than the mean pH for Windsor (5.6) or Hamilton (5.8). Statistical analysis showed that the samples collected in Sarnia had a lower range of pH values (minimum of 4.5 and a maximum of 6.1) than the samples from the other two cities. The difference in pH values between the three cities was tested using a One-Way ANOVA. The difference was found to be statistically significant with an F-value of 12.228 and a P-value of 0.0001. This suggests that differences between the cities do exist and that they differ more than can be expected due to chance.

In Sarnia, as was found in the study by van Herk (2007), lower pH values (4.5) were recorded further from the city center. In both cases, lower lichen species richness and fewer nitrophytes were associated with lower, more acidic pH values as was described by Barkman (1958). Areas with high lichen species richness and more nitrophytes had higher pH values (6.1) and were closer to the city center. In Sarnia, this study also found a difference in the average pH of the 'clean' sites and the 'dirty' sites. Species richness decreases in clean sites with decreasing bark pH, as different lichen species aside from the normal community composed of *P. millegrana*, *C. concolor* and *P. aipolia* are found. The decrease in species richness may be due to

these sites still being too polluted for other more sensitive species to survive in, but not polluted enough for the nitrophytes to thrive in.

When the data from Hamilton and Windsor were compared, it was found that Sarnia has lower maximum, minimum and mean pH than Hamilton or Windsor, but similar range to Hamilton. This suggests that some factor is lowering the pH throughout the city, not in a few localized areas.

A negative correlation ($r^2 = -0.72$) was found between substrate pH and lichen species richness at clean sites; the reverse was found at dirty sites ($r^2 = 0.8$). Species richness was lower in clean sites with a mean of 2.9 species compared to dirty sites which had a mean of 3.8 species. Clean sites had a mean pH of 5.6, while dirty sites had a mean pH of 5.4. Species richness values were higher in dirty sites which had a lower bark pH. Samples collected from dirty sites had a greater range in pH values (4.5 – 6) compared to clean sites (5.1 – 6.1).

Determining sensitivity thresholds of lichens for individual chemical variables is not simple, as lichens are undoubtedly responding to a number of contaminants rather than just a single one. While there is some indication that the busiest roads, such as the Bluewater Bridge International border crossing, show lichen species richness and percent cover gradient, there is little to indicate that the overall traffic volume of local roads has a direct localized impact on the lichen communities. It is possible that NOx concentrations generated by the local vehicle traffic may influence lichen communities and possibly bark pH across the entire city but it is very difficult to separate any local impacts from those contaminants resulting from long distance transport. In depth chemical analysis would be necessary to determine the origins of

contaminants found within the thallus and would not be possible for all contaminants (Simonetti, Gariepy and Carignan, 2003).

5.4 Interpretation of Lichen Species Richness Data

While the EMAN protocol was easily applied in Sarnia, the protocols are intended to identify areas of potential concern for further study, not to explain causality. Causality is difficult to determine in part because lichens can respond to a variety of stressors and the dominance of one or more species at a site is not necessarily a function a single factor (*e.g.*, bark pH, SO_2 in ambient air). In this study, the EMAN protocols were used, but additional data were collected in order to assist with the interpretation of spatial tendencies and provide insight into possible causality.

In lichen species mapping studies, once lichen species richness data have been collected, researchers normally attempt to identify spatial differences between sampling sites by using the mean (Loppi, Giordani, Brunialti, and Isocrono, 2002) or maximum (Dillman, Geiser and Brenner, 2005) lichen species richness, percent lichen cover (Case, 1980) and the presence or absence of indicator species (Nimis *et al.*, 2007). All of these indices have been successfully used to assess ambient air quality in Europe and elsewhere. Use of these indices was also explored in this study.

However, in Sarnia, most sites display only slight spatial differences with the exception of Highway 402 and the Bluewater Bridge (Figure 2.1), no potential areas of concern were identified by the IAP, IHI and species richness indices. Percent lichen cover was uniformly low across most of the study area, possibly due to diffuse eutrophication. However, elevated percentage lichen cover was found on trees near Highway 402 and the Bluewater Bridge border crossing. Although indicator species

(*e.g.*, *P. caperata*) have been useful in identifying different air quality zones in other studies (*e.g.*, Nimis *et al.*, 1990), the presence or absence of any one lichen species in Sarnia was not linked with any obvious environmental or spatial factor. The large sampling grid and the spatial density of the trees surveyed could show broad spatial differences in lichen community variables which can show the sharp decrease in roadside contaminants that have been detected by instrumental studies (*e.g.*, Stocco, MacNeill, Wang, Xu, Guay, Brook and Wheeler, 2008). Lichen species richness was high along the highway and the BlueWater Bridge border crossing where lichen communities were dominated by nitrophytic species such as *C. concolor*, *P. millegrana* and *P. aipolia.* The areas closest to the Bluewater Bridge and the highway had trees where *P. millegrana* formed a mat over most of the tree trunks. Fewer lichen species were found where *P. millegrana* had high percentage cover.

Since *P. millegrana* covers such a large area of these trees, it displaces other lichen species that may have been able to colonize if more bark area had been available. The extreme dominance of one species of lichen such as with *P. millegrana* can be a sign of disturbance. Apart from this, lichen monitoring has not been able to provide evidence of strong lichen community gradients related to habitat or air quality other than the gradient near the border crossing and the highway in Sarnia. This was unexpected given that clear spatial trends were found in Hamilton and in many other studies.

Uniformity in lichen community composition and presumably air quality across Sarnia is not well reflected in the lichen data collected at the Front Street, Corunna and Lasalle Line air quality monitoring stations. At those sites lichen species richness

values were higher than 93 % of trees elsewhere in Sarnia and percent cover values were low for *P. millegrana* and *C. concolor*. As such, these monitoring stations are located in areas of anomalously high lichen species richness. While causality may be difficult to prove and the species richness may be indicative of good air quality, the conditions at these sites are consistent with a nutrient enrichment (*e.g.*, nitrate loading) hypothesis. For example, the LaSalle Line monitoring station is located in the middle of a cornfield where fertilizers and tillage of old corn stalks may have a nitrification effect on the lichens. A nitrification effect is described by Oksanen *et al.* (1990) in which the presence of fertilizers encouraged the localized growth of nitrophytic species despite the lack of many of those species at other sites. In contrast, the Front Street monitoring station site shows many similarities to another site in the north-western corner of the city which also displayed a species richness value (maximum lichen species richness value of nine) far above the rest of the city. Both of these sites are within ten meters of water, a busy roadway and frequent vehicle parking. However, the Corunna site with a maximum lichen species richness value of 9.8 (see Figure 1.1) is an enigma as the monitoring station is located away from water and from any obvious sources of emissions or nitrification with the exception of a parking lot. Further study and the development of a sampling strategy to detect pollution gradients that exist within city blocks would be needed to quantify the factors that may be influencing the values found at these sites.

Percent lichen cover data were also collected in the hope that they could be used to identify local differences in Sarnia's lichen population. Since certain nitrophytic lichen species (*e.g.*, *C. concolor*) tend to be most abundant in the lowest 0.5 m of the

trees, lichen percentage cover values were determined using the line-intercept and the visual method. Most of the variation noticed when using the two methods was due to the presence of *C. concolor* and *C. efflorescens* on the lower trunk. The visual method was preferred as it was less time consuming and required fewer supplies although it is nominally accurate to ± 10%. While percentage cover did not clearly indicate differences within and between sampling sites, areas where cover values were very high or low are still of potential value as an indicator of nutrient enrichment or sharp differences in air quality (*e.g.*, Hamilton and Windsor).

Little is known about the sensitivity of the lichens in the EMAN suite and it is unclear if any of the species will show a response to the particular conditions that exist in Sarnia. More than half (55 %) or six out of the 11 lichen species identified in Sarnia displayed in Table 4.2 are commonly associated with poor air quality. The remaining 46 % or five out of the eleven species identified in Sarnia are known neutrophytes or possible nitrophytes which have not been mentioned in many studies and whose tolerance of air quality is unknown (Table 4.2). These results are similar to what has been found in other studies in which the lichens deemed as nitrophytes are found in abundance in urban areas, while acidophytic species are not found (van Herk, 2001; van Herk, 2002; Gombert et al., 2004; Lambley et al., 2004; van Herk, 2004). The absence of some species from some sites suggests that the EMAN suite may not include species that have an intermediate sensitivity to SO_2. Presumably, if there had been a few species with intermediate sensitivity, then the IAP values would have proved more useful as a way to identify intra-urban differences in the lichen population. However, few lichen species inhabit Sarnia and all of the specimens

found were identified as belonging to the EMAN suite. This suggests that the EMAN suite may be useful in identifying contaminated areas but may not be as useful for study areas that contain a variety of air quality conditions. *Physcia millegrana* was found to be the most common lichen species in Sarnia and was often present at sites even when other species were not. This suggested that the presence of *P. millegrana* may be a general indicator of poor ambient air quality, although the community grouping should also be identified as it occurs under a wide range of habitat and air quality conditions.

While the IAP method is a useful way to investigate intra-urban differences in lichen communities, it is unclear how best to interpret the IAP values. The inclusion of nitrophytes in IAP calculations raises IAP values and gives the misleading impression that Sarnia's air quality is better than is found in cities where IAP values are low (*e.g.*, Hamilton). Though the IAP equation attempts to account for the local rarity of the lichen species, it does not directly take into account the air quality sensitivity of any species. Thus, if all or most species in the study area are nitrophytes the significance of having these species must be evaluated on broader grounds.

Approximately 25 % of Sarnia's IAP values are low (between 0 and 6), suggesting areas of potential concern linked to air and habitat, while only 5 % of the city had IAP values suggestive of fair ambient air quality (IAP values >12). All other sites were classified as having medium IAP values (between 7 and 12) based on natural breaks in the data. Further investigation showed the sites with high lichen species richness values were located in the northwest sector of the study area near the St. Clair River and the border crossing. This region also had high percent lichen

cover. The air quality of this area may have benefited from local wind patterns and their location in a designated green space.

The presence of a population dominated by nitrophytes and the lack of lichen deserts suggests that the lichens are responding to a condition present throughout the city rather than any measurable gradients such as habitat or land use differences. These findings highlighted the need to explore the relationship between lichen species richness and ambient air quality through a possible link between nitrophytes and habitat and bark chemistry.

This study has also explored the possibility of relationships between habitat, land use and lichen communities using variables found to greatly influence lichen distribution (*e.g.*, The Index of Human Impact, Gombert *et al.*, 2004), canopy cover (Pharo and Vitt, 2000), distance to water (Giordani, 2007) and the distance to the nearest road and the volume of traffic (Gombert *et al.*, 2004)).

GIS was found to be an invaluable tool with many uses. Since it displays spatial relationships it can assist in the identification of areas of potential concern and can then be used to help build hypotheses and to guide subsequent sampling. In addition, since attempts to statistically correlate the data collected in this study with lichen species data returned low correlations, GIS provided evidence of a spatial relationship between lichen community variables and the presence of the Bluewater Bridge and the Highway 402 leading to the bridge. However, few trees grow near these sites and the location is not well suited to biomonitoring with lichens. Thus, it was not possible to use the existing trees to provide much insight into the spatial or chemical characteristics of the contaminant inputs at these sites. The finding that lichen species

richness was higher along the highway and near the bridge suggests that sharp air quality gradients may exist in this area. These gradients have been documented as extending approximately 300 m from the highway (Diamond and Parker, 2004). Such a narrow corridor of pollution would easily be missed if suitable trees were not located nearby in large numbers radiating from the zone. Further, the lichen species that are in the EMAN suite may not provide a clear indication of the magnitude of some forms of air pollution. While the many nitrophytes in the EMAN suite may be the only species found near busy roads, one lesson learned in Sarnia is that a simple count of species richness may not give a clear indication of SO_2 in ambient air. Little trust can be placed in IAP values as an indicator of air quality along busy roadways since many of these sites had many nitrophytic species and very rarely had species that are known to be especially sensitive to SO_2 that can out-compete nitrophytes at nitrogen rich sites. This can result in higher IAP values which do not represent the air quality. Under conditions of localized poor air quality, differences in percent lichen cover, differences in the ratios of sensitive to tolerant lichen species and supplementary data examining the chemistry of lichen substrates should all be investigated before attempting to infer air quality conditions in certain settings (*e.g.*, along roadways).

5.5 Summary

This study applied the EMAN lichen biomonitoring methodology and found that it was useful, particularly when combined with GIS, as a preliminary tool for identifying broad spatial trends in lichen populations. GIS enabled the development of hypotheses and guided sampling in addition to providing evidence of a spatial

relationship between lichen community variables and the location of the Bluewater Bridge and Highway 402. In order to properly identify spatial trends using GIS, the sampling density must be considered. The preliminary survey of Sarnia selected four trees per 1 km^2 grid square.

Despite the spatial trend identified by GIS of pockets of high species richness located near the Bluewater Bridge and Highway 402, the majority of sites (92%) were found to have lichen species richness values of less than 11 with low IAP values between 0 and 6. The few sites (8% of all sites surveyed) that had species richness values of less than 11 were found to definitely not be representative of the general conditions in the City of Sarnia where the average lichen species richness value was calculated to be 3.3 species. The average lichen species richness value for the city, based on a survey of 458 trees, is much closer to the trend displayed in Figure 1.1 of the 14 AQI sites. It is possible that the data from the four SLEA and MOE air quality monitoring stations is not representative of the air quality of the rest of the city. This finding highlights how care must be taken to select enough sites to fully represent the conditions present in the desired study area. The uniformity of percent lichen cover (with no lichen deserts) in Sarnia in addition to lichen species richness, suggests that ambient air quality may differ little across the city at the 1 km^2 grid square scale.

All of the lichen species identified in Sarnia were listed in the EMAN suite (Brodo and Craig, 2001) and all were either known or suspected nitrophytes or neutrophytes. Even the two species found to be rare when compared to other species found in Sarnia, *P. detersa* (found on 4% of all trees surveyed) and *M. subaurifera* (found on 3% of all trees surveyed), are known nitrophytes. No acidophytic species

were found. The most common lichen community grouping was found to be comprised of three well-known nitrophytes, *P. millegrana, C. concolor* and *P. aipolia.* This community was found on 46% of all trees surveyed. Since nitrophytes are known to prefer conditions that may have poorer than average air quality with high concentrations of contaminants, the number of nitrophytic species versus acidophytic species may be a better indicator of the air quality than IAP. A large number of nitrophytic species may distort IAP values, resulting in high values that do not reflect the air quality conditions of the study area.

While lichen sensitivity to specific contaminants is difficult to assess, the analysis of bark pH may assist in indentifying areas of concern related to ambient air quality. The pH of tree bark was sampled from sixty trees in Sarnia in order to assess whether the pH of the substrate could be influencing the number and type of lichen species. Like species richness, pH was found to decrease with increasing distance from the city center. The bark collected from trees in Sarnia was found to have a mean pH of 5.5 with a lower range of pH values than those collected from Hamilton or Windsor. The spatial distribution of pH and the proximity to sites with a known source of contamination indicates that there may be a relationship between air quality, pH and lichen community variables. A negative correlation ($r^2 = -0.72$) was found between pH and lichen species richness for sites that had no known source of contamination. The opposite was found to be true for "dirty sites" with a known source of contamination; a positive correlation ($r^2 = 0.8$) was found. The mean species richness at "clean sites" was 2.9 species which is lower than at "dirty sites" which had a mean

of 3.8 species. This suggests that there may be a link between local sources of contamination, pH and lichen species richness.

5.6 Suggested Research Topics and Methodologies

This study found that a link may exist between lichen community, pH and the presence of a source of contamination. Further study, possibly using chemical analysis of tree bark may be capable of providing further insight into this relationship. The lichen survey method used in this study was not meant to assess causality, so there is no indication of whether the pH and therefore the lichen community is being influenced by the presence of a single contaminant or by a mixture of contaminants. Chemical analysis of bark samples and factor analysis may be helpful in isolating which contaminants have the greatest impact. This can be taken further to assess the sensitivity of the various lichen species to different levels of pH and contaminant concentrations. Information such as this could be invaluable to lichen survey studies as it may highlight the existence of indicator species of lichens. The presence or absence of an indicator species can provide information on the environmental quality of a site. If a species is known to be a particularly good indicator of a particular type of contaminant then it may be used instead of an extensive electronic monitoring network, *e.g.*, Spain (Barbero; Hladun; Navarro-Rosinas; Munoz; Arino and Gomez-Bolea, 1998) and the Netherlands (van Herk and ter Braak, 2007).

This study found that bark pH has the potential to identify areas of concern linked to ambient air quality. In order to fully explore this relationship, it is recommended that the pH range at which all identified lichen species are found at be assessed. This may assist in the identification of indicator species.

A more in-depth study could examine intraspecies genetic variation and how it relates to pollution sensitivities similar to a study by Crespo *et al.* (1999). While this study examined the sensitivity of different genotypes of *P. sulcata* to SO_2 concentration, future studies could be done for other lichen species and other contaminants, such as nitrogen. This could assist in determining how comparable lichen species community variables really are between different study areas.

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Appendix I
Survey Form

This survey form was filled out for each tree surveyed. Measurements such as canopy cover and whether a bark sample was collected were recorded in the notes section at the bottom of each section. These forms were used for the initial survey, the main survey and in the collection of bark samples.

Date:_____ **Monitors:**_____

UTM 17T _____ _____ **Street Address** _____

Tree

Reference #:_____ Species:_____
Diameter:_____
Location: Less than 5m from:
☐ major street (4 lanes) ☐ road (2 lanes) ☐ residence ☐ parking lot ☐ factory ☐ field
☐ playground/park ☐ bus stop ☐ other trees

Lichen Species

☐ C. concolor ☐ C. efforescens ☐ F. caperata ☐ G. scripta ☐ M. subaurifera
☐ P. sulcata ☐ P. rubropulchra ☐ P. adscendens ☐ P. aipolia ☐ P. millegrana
☐ P. chloantha ☐ P. detersa ☐ P. rudecta ☐ X. fallax
☐ unknown ☐ sample sample number:_____
☐ die off species:_____
Notes :

Appendix II: Habitat Data

Diameter refers to the diameter of the surveyed tree at chest height. Canopy cover was calculated by subtracting the difference in lux of light recorded by a light meter under the canopy from outside of the canopy. The distance from the lake and river was calculated using a topographic map to estimate the straight-line distance of the site to the nearest body of water. The road category variable relates to Table 3.1. This data was collected during the main survey.

Site	Diameter (cm)	Canopy Cover (Difference)	Distance from Lake (km)	Distance from River (km)	Distance from Road (m)	Road Category
2a	38	30.7	0.2	6	1.1	1
2b	38	21.6	0.2	6	3.8	1
2c	53	26.6	0.2	6	2.4	1
2d	42	51.2	0.2	6	3.9	1
3a	41	7.4	0.75	7	4.3	1
3b	26.5	16.1	0.2	7	7.1	1
3c	53	20.9	0.2	7	6.0	1
3d	32	12.9	0.5	7	7.6	1
4a	52	17.7	0.5	8	5.5	1
4b	23.4	11	0.5	8	3.4	1
4c	37	4.3	0.75	8	6.1	1
4d	36	7.7	0.75	8	6.5	1
5a	63	2.4	0.75	9	5.8	1
5b	23	1.5	0.75	9	5.7	1
5c	35	22.1	0.2	9	5.6	3
5d	28	20.9	0.2	9	9.5	3
6a	54	3.1	0.2	2.5	5.7	1
6b	33.4	4.7	0.2	2.5	0.9	3
6c	43	5.2	0.2	2.5	3.7	3
6d	58.5	7.7	0.2	2.5	7.9	3
7a	38	3.4	0.2	3	3.0	1
7b	34	89.0	0.5	3	1.9	1
7c	45	2.9	0.5	3	6.3	2
7d	41	3.9	0.75	3	7.9	1
8a	29.5	3.3	0.75	4	6.5	1
8b	47	5.3	0.75	4	0.6	1
8c	75	2.5	1	4	6.0	1
8d	48	10.4	1	4	6.1	1
9a	43	4.7	0.75	5	5.9	1
9b	39	3.2	0.75	5	6.6	1
9c	54	3.1	1	5	7.9	3
9d	52.5	3.0	1	5	6.1	1
10a	37.3	2.2	0.75	6	4.3	1
10b	42.4	2.0	0.75	6	4.3	1
10c	34	3.1	0.75	6	6.9	1
10d	37	3.1	1	6	1.9	1
11a	32	7.9	1.75	7	1.6	2
11b	29	9.9	1.75	7	1.8	2
11c	26	11.7	1.75	7	1.4	2

Site	Diameter (cm)	Canopy Cover (Difference)	Distance from Lake (km)	Distance from River (km)	Distance from Road (ft)	Road Category
11d	36.5	13.6	1.75	7	1.8	2
12a	50.2	7.5	2	8	1.2	2
12b	60.2	2.2	2	8	1.2	2
12c	68.7	3.2	2	8	1	2
13a	48	4.7	0.2	0.5	0.9	1
13b	45	3.1	0.2	0.5	1.5	1
13c	41	6.8	0.2	0.5	1.4	1
13d	44	7.0	0.2	0.5	1.6	1
14a	41.5	7.6	0.75	1	1.6	1
14b	47.4	25.4	0.75	1	1.3	1
14c	41	6.4	0.75	1	1.4	1
14d	36.5	5.8	0.75	1	0.9	1
15a	55.9	9.3	1	2	1.4	1
15b	41	8.0	1	2	1.0	1
15c	44	11.7	1	2	1.1	1
15d	70.3	11	1	2	1.8	1
16a	64.5	2.2	1	3	1.6	3
16b	50	3.8	1	3	1.3	3
16c	49	3.5	1	3	1.3	1
16d	44.9	4.2	1	3	1.2	1
17a	55	6.0	2.25	4	1.4	1
17b	48.3	3.4	2.25	4	1.2	1
17c	42.1	4.1	2.25	4	1.1	1
17d	55	8	2.25	4	1.4	1
18a	40	3.2	2.5	5	1.0	1
18b	46	3.6	2.5	5	1.2	1
18c	49.9	14.3	2.5	5	1.3	1
18d	44	3.2	2.5	5	1.1	1
19a	32	6.1	2.5	6	0.8	2
19b	89	6.8	2.5	6	2.3	2
19c	76	12.0	2.5	6	1.9	2
19d	54	3.5	2.5	6	1.4	2
20a	35	2.5	2.75	7	0.9	2
20b	26.5	3.9	2.75	7	0.7	2
20c	31	5.7	2.75	7	0.8	2
20d	44	5.4	2.75	7	1.1	2
23a	44.5	8.1	1.25	1	2.7	1
23b	33.5	7.2	1.25	1	2.6	1
23c	44	79.8	1.25	1	2.6	1
23d	37.5	6.6	1.25	1	1.8	1
24a	37	7.1	2	2	5.5	3
24b	32.5	92.6	2	2	5.9	3
24c	37.9	35.7	2	2	6.2	3
24d	37.2	15.5	2	2	6.2	3
25a	39	18.2	2.5	3	7.9	1
25b	31	4.8	2.5	3	2.2	1
25c	32	11.5	2.5	3	1.8	1
25d	45.4	15.4	2.5	3	8.9	1

Site	Diameter (cm)	Canopy Cover (Difference)	Distance from Lake (km)	Distance from River (km)	Distance from Road (m)	Road Category
26a	38	4.9	3	4	1.3	1
26b	33.8	14.4	3	4	1.3	1
26c	37.2	3.8	3	4	7.1	1
26d	34	9.1	3	4	7.9	1
27a	38	8.5	3.5	5	7.8	2
27b	36	7.3	3.5	5	7.1	2
27c	36	11	3.5	5	6.9	2
27d	41.5	12.3	3.5	5	7.4	2
30a	21	9.03	1	1	11.4	1
30b	21.5	5.6	1	1	11.1	1
30c	30	6.1	1	1	9.8	1
30d	38.5	10.9	1	1	10.1	1
31a	41	13.1	2.5	0.5	1.9	1
31b	30.9	9.1	2.5	0.5	n/a in park	1
31c	34.3	19.7	2.5	0.5	3	1
31d	35.8	28.3	2.5	0.5	8.3	1
32a	39.5	70.6	2.75	1	5.3	1
32b	46.6	76.5	2.75	1	5.5	1
32c	36.6	70.6	2.75	1	5.6	1
33a	61.5	66.7	3.75	2	4.5	1
33b	64	47.1	3.75	2	4.6	1
33c	57.1	61.5	3.75	2	6.1	1
33d	64.3	53.3	3.75	2	4.6	1
34a	28.3	3.8	3.75	3	2.5	1
34b	28.9	23	3.75	3	3	1
34c	33	37.6	4	3	7.6	1
34d	43	35.7	4	3	9.5	1
35a	58.6	15.6	4	4	8.9	1
35b	37	18	4	4	8.9	1
35c	31.9	27.5	4	4	9.5	1
35d	33	27.5	4	4	8.9	1
39a	33	41.2	3.5	0.5	1.5	3
39b	31.8	48.4	3.5	0.5	1.5	3
39c	25.5	41.2	3.5	0.5	1.5	3
39d	29.5	62.9	3.5	0.5	1.4	3
40a	58.2	21.2	4	1	2.0	1
40b	58.5	22.1	4	1	2.0	1
40c	53.9	17.1	4	1	2.4	1
40d	69.2	16.6	4	1	5.0	1
41a	55	39.3	4.5	2	5.0	1
41b	60.6	25.5	4.5	2	5.0	1
41c	50.7	32.7	4.5	2	5.2	1
41d	68.2	28.8	4.5	2	5.1	1
42a	29	43.9	4.75	3	5.1	2
42b	32	29.6	4.75	3	3.6	1
42c	58.8	13.0	4.75	3	3.6	1
42d	52	14.5	4.75	3	6.3	1

Site	Diameter (cm)	Canopy Cover (Difference)	Distance from Lake (km)	Distance from River (km)	Distance from Road (m)	Road Category
43a	24	10.8	4.75	4	6.7	1
43b	37.7	5.9	4.75	4	7.0	2
43c	32	9.3	4.75	4	6.9	2
44a	33	21.4	5	5	3.6	2
44b	36.2	19.2	5	5	7.5	2
44c	30.3	23.5	5	5	12.7	2
44d	35.9	17.4	5	5	12.7	2
45a	99.1	41.7	5.25	6	1.2	2
45b	72.3	43.8	5.25	6	7.6	2
45c	47.7	38.2	5.25	6	2.3	2
45d	45.8	38.7	5.25	6	3.0	2
47a	61.3	49.1	5.25	1	3.0	1
47b	51.9	21.3	5.25	1	2.5	3
47c	81	11.3	5.25	1	6.3	1
47d	40	13.4	5.25	1	3.8	1
48a	61	13.8	5	2	0.8	1
48b	57.5	13.8	5	2	1.0	1
48c	63.2	4.1	5	2	3.8	3
48d	56	3.6	5	2	0.5	3
49a	53.7	20	5.5	3	2.4	1
49b	49.2	9.9	5.5	3	3.0	1
49c	50	10.1	5.5	3	3.8	1
49d	45	18.6	5.5	3	3.8	1
50a	39.7	21.2	6	4	0.6	1
50b	47.6	9.8	6	4	7.7	1
50c	39.3	13.4	6	4	8.9	1
50d	53.2	20	6	4	9.5	1
51a	39	4.8	6	5	8.6	1
51b	37.3	4.4	6	5	9.1	1
51c	38.2	3.2	6	5	5.7	1
51d	35.1	4.4	6	5	7.7	1
52a	33.5	4.9	6.25	6	6.9	1
52b	34.9	4.8	6.25	6	7.1	1
52c	33.6	3.3	6.25	6	7.0	1
52d	29.2	4.8	6.25	6	1.5	1
55a	39.3	16.6	5.5	1.5	5.1	1
55b	37.1	8.1	5.5	1.5	1.8	1
55c	66.6	56.3	5.5	1.5	5.1	1
55d	44.1	49.7	5.5	1.5	5.1	1
56a	40.5	11.2	6	3	2.0	1
56b	61	16.6	6	3	1.8	1
56c	42.2	6	6	3	2.5	1
56d	38.8	20.4	6	3	2.8	1
57a	42.1	18.8	6.25	4	1.8	1
57b	44.4	22.4	6.25	4	2.0	1
57c	43.8	39.5	6.25	4	1.3	1
57d	34.5	14.1	6.25	4	1.8	1
58a	40.7	28.7	6.5	5	2.3	1

Site	Diameter (cm)	Canopy Cover (Difference)	Distance from Lake (km)	Distance from River (km)	Distance from Road (m)	Road Category
58b	42.8	10.1	6.5	5	2.4	1
58c	41.5	9.1	6.5	5	2.5	1
58d	48.3	9.9	6.5	5	2.6	1
59a	26.2	35.7	7	6	7.6	3
59b	41.6	15	7	6	7.6	3
59c	29.7	13.5	7	6	7.6	3
59d	25.3	18.6	7	6	7.6	3
60a	33.3	9.2	7	7	6.9	1
60b	20.4	5.4	7	7	5.1	1
60c	21.5	7.4	7	7	5.1	1
60d	62.8	20.6	7	7	10.1	3
61a	28.8	28.7	7.5	8	7.1	3
61b	52.4	15.3	7.5	8	11.4	3
61c	40.5	5.8	7.5	8	7.6	3
61d	48.7	22.6	7.5	8	7.6	3
62a	49.6	23.3	7.5	9	5.1	3
62b	48.7	16.1	7.5	9	5.3	3
62c	53.1	44.4	7.5	9	5.3	3

Appendix III: GIS Data

The conversion from UTM coordinates into latitude and longitude was done in accordance to the procedure outline by the Brock University Map Library (2005). The onversion was done using ArcMap. The percent cover data displayed was obtained using the visual method outlined in Chapter Three. The lichen species that covered the greatest surface area of the tree trunk was labelled as the 'dominant' species while the species that covered the second greatest surface area when compared to all other lichen species present was referred to as the '2nd Dominant'. Diameter refers to the diameter of the tree trunk at 1.5 meters in height. The Index of Atmospheric Purity (IAP) uses the presence, absence and abundance of lichens in order to generate an air quality index.

Site	Latitude	Longitude	Dominant	2nd Dominant	Diameter (cm)	Richness	% Cover	IAP
1a	43°57'83"	82°22'25"	P. millegrana	C. concolor	38	3	50%	4.75
1b	43°53'99"	82°22' 23"	P. millegrana	C. concolor	36	3	50%	4.75
1c	43°49'52"	82°22' 25"	P. millegrana	C. concolor	42	3	50%	4.75
1d	43°44'52"	82°22' 12"	P. millegrana	C. concolor	40	2	25%	4.75
1e	43°42'82"	82°22' 12"	P. millegrana	C. concolor	34	2	25%	4.75
1f	43°59'31"	82°22' 03"	P. millegrana	C. concolor	53	3	50%	4.75
1g	43°53'88"	82°22' 02"	P. millegrana	C. concolor	31	3	50%	4.75
1h	43°47'07"	82° 22'01"	P. millegrana	C. concolor	33	2	5%	4.75
2a	43°54'94"	82° 21'47"	P. millegrana	C. concolor	38	3	75%	9
2b	43°54'93"	82° 21'44"	P. millegrana	C. concolor	38	3	50%	9
2c	43°56'10"	82° 21'22"	P. millegrana	C. concolor	35	3	5%	8.997
2d	43°49.71	82° 21'23"	P. millegrana	C. concolor	42	2	5%	8.997
2e	43°43'32"	82° 21'24"	P. millegrana	C. concolor	53	5	95%	8.997
2f	43°03'98"	82° 21' 22"	P. millegrana	C. concolor	50	5	95%	8.997
2g	43°03'77	82° 21' 29"	P. millegrana	C. concolor	37	3	75%	8.997
2h	43°42'91"	82° 21' 45"	P. millegrana	C. concolor	38	3	25%	8.997
3a	43°02'05"	82° 21' 11"	P. millegrana	C. concolor	41	2	5%	4.745
3b	43°03'83"	82° 20' 37"	P. millegrana	C. concolor	39	2	5%	4.745
3c	43°53'75"	82° 20' 55"	P. millegrana	C. concolor	53	2	5%	4.745
3d	43°46'40"	82° 20' 56"	P. millegrana	P.detersa	53	2	5%	4.745
3e	43°59'38"	82° 20' 53"	none	none	26	0	0%	4.745
3f	43°57'04"	82° 20' 48"	P. millegrana	P.detersa	50	2	1%	4.745
3g	43°53'85"	82° 20'45"	P. millegrana	C. concolor	32	3	5%	4.745
3h	43°58'73"	82° 20'38"	none	none	30	0	0%	4.745
4a	43°46'38"	82° 20'26"	P. millegrana	C. concolor	33	2	5%	7.299
4b	43°44'25"	82° 20'26"	P. millegrana	C. concolor	37	3	5%	7.299
4c	43°50'53"	82° 20'24"	C. concolor	P.millegrana	34	2	5%	7.299
4d	43°58'10"	82° 20'22"	P. millegrana	C. concolor	36	2	5%	7.299
4e	43°56'06"	82° 20'1"	C. concolor	P.millegrana	36	3	5%	7.299
4f	43°01'60"	82° 19'56"	P. millegrana	C. concolor	52	3	5%	7.299
4g	43°58'94"	82° 20'01"	P. millegrana	C. concolor	23	4	25%	7.299
5a	43°10'84"	82° 19'34"	P. millegrana	C. concolor	24	2	1%	3.930
5b	43°11'47"	82° 19'21"	P. millegrana	none	35	1	1%	3.930

Site	Latitude	Longitude	Dominant	2nd Dominant	Diameter (cm)	Richness	% Cover	IAP
5c	43°09'76"	82° 19'15"	P. millegrana	none	28	1	1%	3.930
5d	43°06'15"	82° 19'17"	P. millegrana	none	30	1	1%	3.930
5e	43°00'63"	82° 19'50"	P. millegrana	C. concolor	40	2	5%	3.930
5f	43°04'67"	82° 19'42"	P. millegrana	C. concolor	63	2	5%	3.930
5g	43°06'58"	82° 19'37"	P. millegrana	C. concolor	23	2	5%	3.930
6a	43°27'71"	82° 23'36"	P. millegrana	C. concolor	54	5	25%	11.350
6b	43°22'06"	82° 23'44"	P. millegrana	C. concolor	33	2	1%	11.350
6c	43°15'88"	82° 23'37"	P. millegrana	C. concolor	43	6	50%	11.350
6d	43°17'80"	82° 23'30"	P. millegrana	C. concolor	58.	4	50%	11.350
6e	43°31'86"	82° 23'16"	P. millegrana	C. concolor	53	4	50%	11.350
6f	43°29'51"	82° 23'14"	P. millegrana	C. concolor	37	5	50%	11.350
6g	43°19'93"	82° 23'20"	P. millegrana	C. concolor	36	5	50%	11.350
6h	43°19'50"	82° 23'15"	P. millegrana	C. concolor	33	3	25%	11.350
7a	43°37'92"	82° 22'9"	P. millegrana	C. concolor	38	3	5%	11.350
7b	43°30'89"	82° 22'55"	P. millegrana	C. concolor	34	6	50%	11.350
7c	43°29'39"	82° 22'36"	P. millegrana	C. concolor	45	5	75%	11.350
7d	43°29'61"	82° 22'43"	P. millegrana	C. concolor	40	5	75%	11.350
7e	43°37'59"	82° 22'36"	P. millegrana	C. concolor	40	4	75%	11.350
7f	43°34'62"	82° 22'57"	P. millegrana	C. concolor	41	3	25%	11.350
7g	43°26'84"	82° 22'55"	P. millegrana	C. concolor	38	3	25%	11.350
7h	43°41'32"	82° 23'03"	P. millegrana	C. concolor	39	4	50%	11.350
8a	43°34'17"	82° 22'16"	P. millegrana	C. concolor	29	2	5%	9.688
8b	43°32'04"	82° 22'15"	P. millegrana	C. concolor	47	2	5%	9.688
8c	43°27'78"	82° 22'05"	P. millegrana	C. concolor	34	2	5%	9.688
8d	43°40'99"	82° 22'15"	P. millegrana	C. concolor	45	2	5%	9.688
8e	43°25'55"	82° 22'18"	P. millegrana	C. concolor	49	5	50%	9.688
8f	43°20'34"	82° 22'19"	P. millegrana	C. concolor	61	5	50%	9.688
8g	43°27'14"	82° 21'57"	P. millegrana	C. concolor	75	5	50%	9.688
8h	43°21'73"	82° 22'24"	P. millegrana	C. concolor	48	5	50%	9.688
9a	43°40'86"	82° 21'32"	P. millegrana	C.efflorescens	43	4	50%	11.690
9b	43°31'17"	82° 21'46"	P. millegrana	C. concolor	39	3	25%	11.690
9c	43°32'02"	82° 21'40"	P. millegrana	P. aipolia	54	7	95%	11.690
9d	43°23'09"	82° 21' 37"	P. millegrana	C. efflorescens	52	3	50%	11.690
9e	43°25'96"	82° 21' 31"	P. millegrana	C. efflorescens	50	3	25%	11.690
9f	43°40'96"	82° 21' 37"	P. millegrana	C. concolor	51	3	25%	11.690
9g	43°36'18"	82° 21' 21"	P. millegrana	P. aipolia	50	6	95%	11.690
10a	43°40'44"	82° 21' 07"	P. millegrana	C. concolor	34	5	75%	9.453
10b	43°34'25"	82° 20' 50"	P. millegrana	C. concolor	33	5	75%	9.453
10c	43°27'53"	82° 20' 50"	P. millegrana	P. aipolia	37	2	5%	9.453
10d	43°28'71"	82° 20' 47"	P. millegrana	P. aipolia	42	3	5%	9.453
10e	43°21'15"	82° 21' 08"	P. millegrana	P. aipolia	37	4	5%	9.453
10f	43°23'49"	82° 21' 08"	P. millegrana	P. aipolia	36	4	5%	9.453
10g	43°13'80"	82°° 21 07	P. millegrana	P. aipolia	40	3	5%	9.453
10h	43°40'32"	82° 20' 45"	P. millegrana	P. aipolia	38	4	5%	9.453
11a	43°13'79"	82° 20' 27"	P. aipolia	P. millegrana	32	4	5%	7.992

Site	Latitude	Longitude	Dominant	2nd Dominant	Diameter (cm)	Richness	% Cover	IAP
11b	43°13'68"	82° 20' 21"	P. aipolia	C. concolor	29	4	5%	7.992
11c	43°14'32"	82° 20' 16"	P. aipolia	C. concolor	30	4	5%	7.992
11d	43°28'69"	82° 20' 10"	P. aipolia	P. millegrana	32	4	25%	7.992
11e	43°24'00"	82° 20' 10"	P. aipolia	P. millegrana	31	4	25%	7.992
11f	43°29'86"	82° 20' 15"	P. millegrana	P. aipolia	28	4	25%	7.992
11g	43°23'58"	82° 20' 14"	P. millegrana	P. aipolia	27	4	5%	7.992
11h	43°27'41"	82° 20' 14"	P. millegrana	C. concolor	36	2	5%	7.992
12a	43°21'77"	82° 19' 49"	P. millegrana	C. concolor	34	2	1%	6.038
12b	43°16'14"	82° 19' 49"	P. millegrana	C. concolor	29	2	1%	6.038
12c	43°30'51"	82° 19' 33"	P. millegrana	C. concolor	30	2	1%	6.038
12d	43°32'74"	82° 19' 32"	P. millegrana	C. concolor	32	2	1%	6.038
12e	43°37'03"	82 19' 30"	P. millegrana	C. concolor	44	3	5%	6.038
12f	43°39'70"	82 19' 27"	P. millegrana	C. concolor	37	3	5%	6.038
13a	43°02'83"	82 24' 53"	P. millegrana	P. sulcata	48	4	50%	19.680
13b	43°01'82"	82 24' 50"	P. millegrana	C. concolor	45	7	50%	19.680
13c	43°01'58"	82 24' 47"	P. millegrana	C. concolor	40	7	75%	19.680
13d	42°57'74"	82 25' 02"	P. millegrana	C. concolor	44	9	95%	19.680
13e	42°55'09"	82 25' 04"	P. millegrana	C. concolor	43	9	95%	19.680
13f	42°53'09"	82 24' 48"	P. millegrana	C. concolor	39	7	95%	19.680
13g	42°52'61"	82 24' 41"	P. millegrana	P. adscendens	41	7	75%	19.680
13h	42°50'77"	82° 24' 34"	P. millegrana	P. adscendens	39	7	75%	19.680
14a	42°50'45"	82° 24' 28"	P. millegrana	C. concolor	41	5	25%	9.316
14b	42°50'06"	82° 24' 24"	P. millegrana	C. concolor	47	5	25%	9.316
14c	42°50'18"	82° 24' 20"	P. millegrana	C. concolor	46	5	50%	9.316
14d	43°04'54"	82° 24' 05"	P. millegrana	C. concolor	47	5	25%	9.316
14e	43°05'18"	82° 24' 00"	P. millegrana	C. concolor	38	3	5%	9.316
14f	43°02'38"	82° 24' 23"	P. millegrana	C. concolor	41	3	5%	9.316
14g	42°50'54"	82° 24' 09"	P. millegrana	none	36	1	5%	9.316
14h	42°50'30"	82° 24' 03"	P. millegrana	none	35	1	5%	9.316
15a	42°52'10"	82° 23' 39"	P. millegrana	C. concolor	55	6	75%	12.420
15b	42°52'97"	82° 23' 29"	P. millegrana	C. concolor	54	6	75%	12.420
15c	42°56'03"	82° 23' 28"	P. millegrana	C. concolor	41	5	50%	12.420
15d	43°10'20"	82° 23' 42"	P. millegrana	C. concolor	43	5	75%	12.420
15e	43°09'43"	82° 23' 37"	P. millegrana	C. concolor	44	4	50%	12.420
15f	43°02'13"	82° 23' 19"	P. millegrana	C. concolor	70	4	50%	12.420
15g	42°56'68"	82° 23' 19"	P. millegrana	C. concolor	67	4	50%	12.420
15h	42°59'62"	82° 23' 28"	P. millegrana	C. concolor	66	4	50%	12.420
16a	43°00'05"	82° 23' 07"	P. millegrana	C. efflorescens	64	5	50%	9.881
16b	42°50'92"	82° 22' 45"	P. millegrana	C. efflorescens	63	5	75%	9.881
16c	42°50'88"	82° 22' 42"	P. millegrana	C. concolor	50	4	25%	9.881
16d	42°53'42"	82° 22' 45"	C. concolor	P. millegrana	36	3	5%	9.881
16e	43°03'75"	82° 22' 54"	C. concolor	P. millegrana	49	4	25%	9.881
16f	43°09'20"	82° 23' 05"	P. millegrana	C. concolor	49	4	25%	9.881
16g	42°52'01"	82° 23' 01"	P. millegrana	C. concolor	44	2	5%	9.881
16h	43°11'70"	82° 22' 56"	P. millegrana	C. efflorescens	62	5	25%	9.881

Site	Latitude	Longitude	Dominant	2nd Dominant	Diameter (cm)	Richness	% Cover	IAP
17a	42°51'25"	82° 22' 17"	P. millegrana	C. efflorescens	55	5	25%	9.422
17b	42°57'12"	82° 22' 00"	P. millegrana	C. concolor	48	2	25%	9.422
17c	42°50'92"	82° 22' 25"	P. millegrana	C. efflorescens	50	5	25%	9.422
17d	43°11'15"	82° 22' 13"	P. millegrana	C. concolor	53	3	25%	9.422
17e	43°11'27"	82° 22' 20"	P. millegrana	C. concolor	55	3	25%	9.422
17f	43°03'76"	82° 22' 06"	C. concolor	P. millegrana	42	4	25%	9.422
17g	43°02'89"	82° 22' 16"	C. concolor	P. millegrana	40	4	25%	9.422
17h	42°50'81"	82° 22' 25"	P. millegrana	C. concolor	50	3	25%	9.422
18a	43°02'24"	82° 21' 38"	P. millegrana	C. concolor	40	3	25%	7.960
18b	43°09'86"	82° 21' 19"	P. millegrana	C. concolor	46	2	5%	7.960
18c	43°10'84"	82° 21' 42"	P. millegrana	C. concolor	49	4	25%	7.960
18d	43°03'65"	82° 21' 29"	P. millegrana	C. concolor	45	4	25%	7.960
18e	43°10'51"	82° 21' 38"	P. millegrana	C. concolor	41	3	25%	7.960
18f	42°59'29"	82° 21' 27"	P. millegrana	P. aipolia	44	3	5%	7.960
18g	42°54'06"	82° 21' 27"	P. millegrana	P. aipolia	42	3	5%	7.960
18h	43°10'51"	82° 21' 36"	P. millegrana	P. aipolia	39	3	5%	7.960
19a	43°05'91"	82° 20' 52"	P. millegrana	P. aipolia	32	3	5%	5.287
19b	43°06'61"	82° 20' 46"	P. millegrana	P. aipolia	89	2	5%	5.287
19c	43°08'10"	82° 20' 39"	P. millegrana	P. aipolia	76	1	1%	5.287
19d	42°59'75"	82° 21' 04"	P. millegrana	P. aipolia	54	2	5%	5.287
19e	43°08'50"	82° 21' 06"	P. millegrana	P. aipolia	44	2	5%	5.287
19f	42°43'03"	82° 21' 00"	P. millegrana	P. aipolia	37	2	5%	5.287
19g	42°48'89"	82° 20' 43"	P. millegrana	P. aipolia	70	3	5%	5.287
19h	42°49'49"	82° 20' 42"	P. millegrana	P. aipolia	66	3	25%	5.287
20a	43°10'36"	82° 19' 58"	P. millegrana	P. aipolia	31	5	50%	9.022
20b	43°02'91"	82° 20' 32"	P. aipolia	P. millegrana	35	4	25%	9.022
20c	42°59'53"	82° 20' 31"	P. aipolia	P. millegrana	33	4	25%	9.022
20d	43°09'18"	82° 20' 23"	P. millegrana	P. aipolia	30	5	25%	9.022
20e	43°09'77"	82° 20' 15"	P. millegrana	none	29	1	5%	9.022
20f	42°51'55"	82° 20' 05"	P. millegrana	none	26	1	5%	9.022
21a	42°42'89"	82° 19' 50"	P. millegrana	none	39	1	5%	2.616
21b	42°43'58"	82° 19' 44"	P. millegrana	none	37	1	1%	2.616
21c	43°05'06"	82° 19' 30"	P. millegrana	none	41	1	1%	2.616
21d	42°59'89"	82° 19' 28"	P. millegrana	none	26	1	1%	2.616
21e	43°05'15"	82° 19' 21"	P. millegrana	none	34	1	1%	2.616
21f	43°05'75"	82° 19' 18"	P. millegrana	none	33	1	1%	2.616
21g	42°42'78"	82° 19' 44"	P. millegrana	none	27	1	5%	2.616
22a	42°38'94"	82° 24' 40"	P. millegrana	P. aipolia	40	5	25%	13.210
22b	42°39'74"	82° 24' 31"	P. millegrana	P. aipolia	45	5	25%	13.210
22c	42°40'04"	82° 24' 35"	P. millegrana	P. aipolia	39	6	25%	13.210
22d	42°24'24"	82° 24' 50"	P. millegrana	P. aipolia	36	5	50%	13.210
22e	42°24'44"	82° 24' 44"	P. millegrana	P. aipolia	41	5	50%	13.210
22f	42°30'03"	82° 24' 47"	P. millegrana	P. aipolia	42	6	50%	13.210
22g	42°42'90"	82° 24' 58"	P. millegrana	P. sulcata	38	6	25%	13.210

22h	42°38'39"	82° 24' 36"	P. millegrana	P. sulcata	36	6	25%	13.210
23a	42°26'50"	82° 24' 18"	P. millegrana	P. sulcata	44	6	25%	16.200
23b	42°29'84"	82° 24' 15"	P. millegrana	P. sulcata	43	6	75%	16.200
23c	42°39'74"	82° 24' 16"	P. millegrana	P. aipolia	33	6	75%	16.200
23d	42°42'18"	82° 24' 10"	P. millegrana	P. aipolia	44	6	75%	16.200
23e	42°30'67"	82° 24' 07"	P. millegrana	P. aipolia	41	6	75%	16.200
23f	42°30'29"	82° 24' 02"	P. millegrana	P. aipolia	42	6	75%	16.200
23g	42°32'47"	82° 23' 59"	P. millegrana	F. caperata	37	7	95%	16.200
23h	42°37'03"	82° 23' 59"	P. millegrana	F. caperata	35	7	95%	16.200
24a	42°39'18"	82° 23' 14"	P. millegrana	X. fallax	37	4	75%	13.210
24b	42°37'83"	82° 23' 11"	P. millegrana	X. fallax	32	4	75%	13.210
24c	42°31'37"	82° 23' 22"	P. millegrana	X. fallax	37	4	75%	13.210
24d	42°27'65"	82° 23' 44"	P. millegrana	X. fallax	36	4	50%	13.210
24e	42°31'70"	82° 23' 44"	P. millegrana	X. fallax	33	4	50%	13.210
24f	42°36'64"	82° 23' 46"	P. millegrana	X. fallax	31	5	50%	13.210
24g	42°37'85"	82° 23' 24"	P. millegrana	P. aipolia	37	5	50%	13.210
24h	42°25'73"	82° 23' 22"	P. millegrana	P. aipolia	36	5	50%	13.210
25a	42°26'62"	82° 22' 44"	P. millegrana	P. aipolia	31	4	50%	10.780
25b	42°25'21"	82° 22' 46"	P. millegrana	P. aipolia	32	3	25%	10.780
25c	42°21'63"	82° 22' 47"	P. millegrana	P. aipolia	34	3	25%	10.780
25d	42°31'10"	82° 22' 50"	P. millegrana	C. efflorescens	39	4	25%	10.780
25e	42°21'43"	82° 22' 54"	P. millegrana	P. aipolia	32	3	50%	10.780
25f	42°38'46"	82° 22' 58"	P. millegrana	C. concolor	44	5	50%	10.780
25g	42°21'30"	82° 23' 04"	P. millegrana	C. concolor	42	5	50%	10.780
25h	42°20'92"	82° 22' 55"	P. millegrana	C. efflorescens	36	4	25%	10.780
26a	42°19'50"	82° 22' 06"	P. millegrana	C. efflorescens	38	5	25%	12.530
26b	42°24'49"	82° 22' 06"	P. millegrana	C. efflorescens	36	4	25%	12.530
26c	42°28'59"	82° 22' 05"	P. millegrana	C. concolor	33	6	50%	12.530
26d	42°29'94"	82° 22' 12"	P. millegrana	C. concolor	31	6	50%	12.530
26e	42°32'70"	82° 22' 26"	P. millegrana	C. concolor	34	4	50%	12.530
26f	42°22'77"	82° 22' 26"	P. millegrana	C. concolor	35	4	25%	12.530
26g	42°29'24"	82° 22' 28"	P. millegrana	C. concolor	37	3	25%	12.530
26h	42°32'70"	82° 22' 27"	P. millegrana	C. concolor	33	3	25%	12.530
27a	42°16'81"	82° 21' 44"	P. aipolia	P. millegrana	38	4	25%	11.590
27b	42°21'03"	82° 21' 44"	P. aipolia	P. millegrana	41	4	25%	11.590
27c	42°36'28"	82° 21' 46"	P. aipolia	P. millegrana	36	4	25%	11.590
27d	42°40'07"	82° 21' 40"	P. millegrana	P. aipolia	38	4	25%	11.590
27e	42°36'15"	82° 21' 32"	P. millegrana	P. aipolia	36	6	75%	11.590
28a	42°40'58"	82° 21' 06"	P. adscendens	P. millegrana	27	2	5%	7.358
28b	42°41'29"	82° 21' 02"	P. millegrana	P. adscendens	33	2	5%	7.358
28c	42°34'09"	82° 21' 06"	P. millegrana	P. adscendens	31	2	1%	7.358
28d	42°13'90"	82° 21' 08"	P. millegrana	P. adscendens	26	2	1%	7.358
28e	42°17'50"	82° 21' 12"	P. millegrana	P. adscendens	23	2	5%	7.358
28f	42°31'77"	82° 20' 46"	P. millegrana	P. aipolia	31	3	5%	7.358
29a	42°19'21"	82° 20' 01"	P. adscendens	P. aipolia	45	4	25%	8.751
29b	42°22'27"	82° 20' 07"	P. adscendens	P. aipolia	33	4	25%	8.751

29c	42°12'94"	82° 20' 15"	P. aipolia	P. adscendens	37	3	25%	8.751
29d	42°12'66"	82° 20' 21"	P. aipolia	P. adscendens	29	3	25%	8.751
29e	42°29'44"	82° 20' 07"	P. millegrana	P. aipolia	29	3	5%	8.751
29f	42°29'66"	82° 20' 12"	P. adscendens	P. aipolia	26	3	5%	8.751
29g	42°32'57"	82° 20' 15"	P. millegrana	P. aipolia	23	3	5%	8.751
29h	42°18'91"	82° 20' 01"	P. adscendens	P. aipolia	33	4	5%	8.751
30a	42°40'38"	82° 19' 25"	P. aipolia	P. adscendens	21	5	5%	12.769
30b	42°38'21"	82° 19' 18"	P. millegrana	P. aipolia	21	5	5%	12.769
30c	42°23'70"	82° 19' 36"	P. millegrana	P. aipolia	30	5	25%	12.769
30d	42°39'85"	82° 19' 53"	P. adscendens	P. aipolia	23	5	25%	12.769
30e	42°33'04"	82° 19' 44"	P. aipolia	P. millegrana	38	4	5%	12.769
30f	42°29'93"	82° 19' 45"	P. aipolia	P. millegrana	34	3	5%	12.769
30g	42°22'56"	82° 19' 50"	P. aipolia	P. millegrana	31	3	5%	12.769
30h	42°21'62"	82° 19' 35"	P. aipolia	P. millegrana	27	3	5%	12.769
31a	42°51'05"	82° 24' 24"	P. millegrana	P. aipolia	41	3	25%	7.358
31b	42°53'59"	82° 24' 24"	P. millegrana	P. aipolia	30	2	5%	7.358
31c	42°51'64"	82° 24' 13"	P. millegrana	P. aipolia	34	3	25%	7.358
31d	42°53'71"	82° 24' 13"	P. millegrana	P. aipolia	35	3	25%	7.358
31e	42°47'74"	82° 23' 59"	P. millegrana	P. aipolia	31	3	25%	7.358
31f	42°52'68"	82° 24' 04"	P. millegrana	P. aipolia	29	3	25%	7.358
31g	42°04'88"	82° 24' 08"	P. millegrana	P. aipolia	40	2	5%	7.358
31h	42°06'27"	82° 24' 16"	P. millegrana	P. aipolia	44	3	25%	7.358
32a	42°47'04"	82° 23' 17"	P. millegrana	C. concolor	39	4	25%	13.493
32b	42°49'57"	82° 23' 16"	P. millegrana	C. concolor	46	4	50%	13.493
32c	42°58'54"	82° 23' 16"	P. millegrana	P. aipolia	43	4	50%	13.493
32d	42°58'66"	82° 23' 21"	P. millegrana	C. concolor	36	4	50%	13.493
32e	42°59'12"	82° 23' 25"	P. millegrana	C. concolor	32	5	50%	13.493
32f	42°08'55"	82° 23' 40"	P. millegrana	C. concolor	37	5	75%	13.493
32g	42°08'67"	82° 23' 44"	P. millegrana	C. concolor	29	5	75%	13.493
32h	42°50'84"	82° 23' 39"	P. millegrana	C. concolor	41	5	75%	13.493
33a	42°45'42"	82° 22' 40"	P. millegrana	C. concolor	61	3	50%	8.751
33b	42°48'64"	82° 22' 39"	P. millegrana	C. concolor	64	4	50%	8.751
33c	42°49'80"	82° 23' 05"	P. millegrana	C. concolor	57	3	25%	8.751
33d	42°49'68"	82° 22' 59"	P. millegrana	C. concolor	62	4	50%	8.751
33e	42°50'03"	82° 22' 59"	P. millegrana	P. chloantha	64	3	25%	8.751
33f	42°00'50"	82° 22' 51"	P. millegrana	P. chloantha	60	3	25%	8.751
33g	42°10'05"	82° 23' 00"	P. millegrana	C. concolor	54	3	25%	8.751
33h	42°07'40"	82° 23' 00"	P. millegrana	C. concolor	55	4	50%	8.751
34a	42°50'43"	82° 22' 00"	P. millegrana	C. concolor	28	4	50%	11.340
34b	42°51'53"	82° 21' 57"	P. millegrana	C. concolor	28	4	50%	11.340
34c	42°53'94"	82° 21' 57"	P. millegrana	C. concolor	33	4	50%	11.340
34d	42°04'94"	82° 22' 14"	P. millegrana	C. concolor	43	4	50	11.340
34e	42°06'92"	82° 22' 25"	P. millegrana	C. concolor	29	4	50%	11.340
34f	42°50'54"	82° 22' 16"	P. millegrana	C. concolor	31	4	50%	11.340
34g	42°11'09"	82° 22' 23"	P. millegrana	C. concolor	24	4	50%	11.340
34h	42°10'33"	82° 22' 14"	P. millegrana	C. concolor	45	4	25%	11.340

35a	42°49'66"	82° 21' 48"	P. millegrana	C. concolor	58	3	25%	7.294
35b	42°50'54"	82° 21' 43"	P. millegrana	C. concolor	37	3	25%	7.294
35c	42°00'09"	82° 21' 35"	P. millegrana	C. concolor	31	3	25%	7.294
35d	42°06'35"	82° 21' 32"	P. millegrana	C. concolor	33	2	5%	7.294
35e	42°10'85"	82° 21' 39"	P. millegrana	C. concolor	36	2	5%	7.294
35f	42°10'85"	82° 21' 47"	P. millegrana	C. concolor	38	2	25%	7.294
35g	42°49'33"	82° 21' 36"	P. millegrana	C. concolor	42	3	5%	7.294
35h	42°49'43"	82° 21' 32"	P. millegrana	C. concolor	35	3	5%	7.294
36a	42°10'09"	82° 21' 08"	P. millegrana	C. concolor	29	3	5%	7.294
36b	42°10'09"	82° 21' 04"	P. millegrana	C. concolor	38	3	25%	7.294
36c	42°09'54"	82° 20' 59"	P. millegrana	C. concolor	42	3	25%	7.294
36d	42°58'00"	82° 21' 00"	P. millegrana	C. concolor	33	3	25%	7.294
36e	42°52'29"	82° 21'10"	P. millegrana	C. concolor	24	3	5%	7.294
36f	42°56'35"	82° 21'04"	P. millegrana	C. concolor	27	2	5%	7.294
36g	42° 00'74"	82° 21'04"	P. millegrana	C. concolor	34	2	25%	7.294
37a	42°05'48"	82° 20'21"	P. millegrana	C. concolor	29	2	25%	4.208
37b	42°09'11"	82° 20'21"	P. millegrana	C. concolor	27	2	5%	4.208
37c	42°09'78"	82° 20'23"	P. millegrana	C. concolor	33	2	5%	4.208
37d	42°55'78"	82° 20'04"	P. millegrana	none	34	1	5%	4.208
37e	42°43'16"	82° 20'33"	P. millegrana	none	31	1	5%	4.208
38a	42°03'82"	82° 19'50"	P. millegrana	none	25	1	5%	4.208
38b	42°06'91"	82° 19'49"	P. millegrana	C. concolor	33	2	5%	4.208
38c	42°07'23"	82° 19'46"	P. millegrana	C. concolor	34	2	5%	4.208
38d	42°07'12"	82° 19'40"	P. millegrana	none	44	1	5%	4.208
38e	42°46'67"	82° 19'33"	P. millegrana	C. concolor	52	2	1%	4.208
38f	42°47'44"	82° 19'34"	P. millegrana	none	56	1	1%	4.208
39a	42°18'01"	82° 24'01"	P. millegrana	P. aipolia	33	3	25%	11.218
39b	42°39'41"	82° 24'17"	P. millegrana	P. aipolia	31	4	50%	11.218
39c	42°30'75"	82° 24'20"	P. millegrana	P. aipolia	25	3	25%	11.218
39d	42°38'37"	82° 24'14"	P. millegrana	P. aipolia	29	4	25%	11.218
39e	42°28'70"	82° 24'08"	P. millegrana	P. aipolia	30	3	50%	11.218
39f	42°24'39"	82° 24'01"	P. millegrana	P. aipolia	27	4	50%	11.218
39g	42°42'20"	82° 24'15"	P. millegrana	P. aipolia	33	3	25%	11.218
39h	42°24'87"	82° 24'08"	P. millegrana	P. aipolia	32	4	75%	11.218
40a	42°15'29"	82° 23'47"	P. millegrana	C. efflorescens	58	2	25%	4.350
40b	42°14'81"	82° 23'38"	P. millegrana	C. efflorescens	57	2	25%	4.350
40c	42°25'73"	82° 23'47"	P. millegrana	C. efflorescens	56	2	25%	4.350
40d	42°31'66"	82° 23'48"	P. millegrana	none	58	1	25%	4.350
40e	42°35'01"	82° 23'22"	P. millegrana	none	69	1	25%	4.350
40f	42°28'03"	82° 23'21"	P. millegrana	C. concolor	53	3	25%	4.350
40g	42°25'25"	82° 23'21"	P. millegrana	C. concolor	52	3	25%	4.350
40h	42°38'73"	82° 23'45"	P. millegrana	C. concolor	50	3	25%	4.350
41a	42°18'46"	82° 23'08"	P. millegrana	C. concolor	55	3	25%	8.187
41b	42°16'64"	82° 23'10"	P. millegrana	C. concolor	60	3	75%	8.187
41c	42°25'73"	82° 23'07"	P. millegrana	C. concolor	50	4	50%	8.187
41d	42°24'59"	82° 23'01"	P. millegrana	C. concolor	68	3	50%	8.187

41e	42°24'59"	82° 22'43"	P. millegrana	C. concolor	66	4	50%	8.187
41f	42°24'20"	82° 22'38"	P. millegrana	C. concolor	56	4	75%	8.187
41g	42°39'41"	82° 23'03"	P. millegrana	C. concolor	34	3	50%	8.187
41h	42°37'69"	82° 22'46"	P. millegrana	C. concolor	37	3	50%	8.187
42a	42°20'18"	82° 22'27"	P. millegrana	C. concolor	32	3	50%	7.238
42b	42°28'70"	82° 22'24"	P. millegrana	C. concolor	52	3	75%	7.238
42c	42°29'85"	82° 22'15"	P. millegrana	P. aipolia	29	3	75%	7.238
42d	42°35'11"	82° 22'04"	P. millegrana	C. concolor	34	3	50%	7.238
42e	42°35'49"	82° 21'59"	P. millegrana	C. concolor	37	3	50%	7.238
42f	42°40'84"	82° 22'18"	P. millegrana	P. sulcata	58	2	50%	7.238
42g	42°40'46"	82° 22'24"	P. millegrana	P. sulcata	56	2	25%	7.238
42h	42°14'16"	82° 22'13"	P. millegrana	P. aipolia	31	3	25%	7.238
43a	42°21'86"	82° 21'47"	P. aipolia	P. millegrana	37	5	50%	10.514
43b	42°35'06"	82° 21'39"	P. aipolia	P. millegrana	34	5	50%	10.514
43c	42°17'65"	82° 21'48"	P. aipolia	C. concolor	24	2	25%	10.514
43d	42°15'74"	82° 21'42"	P. aipolia	C. concolor	29	2	25%	10.514
43e	42°25'30"	82° 21'27"	P. aipolia	P. millegrana	37	5	50%	10.514
43f	42°27'41"	82° 21'24"	P. aipolia	P. millegrana	32	5	50%	10.514
43g	42°29'03"	82° 21'20"	P. aipolia	P. millegrana	34	5	75%	10.514
43h	42°35'73"	82° 21'26"	P. millegrana	P. aipolia	36	5	75%	10.514
44a	42°27'02"	82° 20'50"	P. millegrana	C. concolor	33	6	95%	10.429
44b	42°24'91"	82° 20'51"	P. millegrana	C. concolor	36	6	75%	10.429
44c	42°18'99"	82° 21'02"	P. millegrana	C. concolor	30	3	25%	10.429
44d	42°36'22"	82° 21'05"	P. millegrana	C. concolor	35	4	25%	10.429
44e	42°35'71"	82° 21'02"	P. millegrana	C. concolor	36	3	25%	10.429
44f	42°28'37"	82° 21'08"	P. millegrana	C. concolor	37	4	25%	10.492
44g	42°15'40"	82° 21'07"	P. millegrana	C. concolor	32	4	25%	10.492
44h	42°15'55"	82° 20'45"	P. millegrana	C. concolor	31	3	25%	10.492
45a	42°25'02"	82° 20'28"	P. millegrana	none	99	1	25%	4.028
45b	42°27'25"	82° 20'22"	P. millegrana	none	72	1	1%	4.028
45c	42°29'66"	82° 20'21"	P. millegrana	none	82	1	1%	4.028
45d	42°19'28"	82° 20'34"	P. millegrana	C. concolor	47	2	1%	4.028
45e	42°18'17"	82° 20'34"	P. millegrana	C. concolor	52	3	5%	4.028
45f	42°16'41"	82° 20'34"	P. millegrana	C. concolor	38	3	5%	4.028
46a	42°39'52"	82° 19'50"	P. millegrana	none	47	1	5%	4.028
46b	42°39'70"	82° 19'44"	P. millegrana	none	43	1	1%	4.028
46c	42°31'24"	82° 19'44"	P. millegrana	none	35	1	1%	4.028
46d	42°13'30"	82° 19'46"	P. millegrana	C. concolor	47	2	1%	4.028
46e	42°16'18"	82° 19'45"	P. millegrana	C. concolor	41	2	1%	4.028
47a	42°12'26"	82° 24'22"	P. millegrana	C. concolor	27	2	5%	5.680
47b	42°56'47"	82° 23'58"	P. millegrana	C. concolor	32	2	5%	5.680
47c	42°53'40"	82° 23'58"	P. millegrana	C. concolor	35	3	5%	5.680
47d	42°46'32"	82° 24'28"	P. millegrana	C. concolor	51	3	1%	5.680
47e	42°57'77"	82° 24'05"	P. millegrana	C. concolor	53	2	1%	5.680
47f	42°52'92"	82° 24'27"	none	none	45	0	0%	5.680
47g	42°12'90"	82° 24'08"	none	none	38	0	0%	5.680

47h	42°58'88"	82° 24'28"	none	none	34	0	0%	5.680
48a	42°57'03"	82° 23'18"	P. millegrana	C. concolor	29	2	1%	4.577
48b	42°08'92"	82° 23'41"	none	none	27	0	0%	4.577
48c	42°52'29"	82° 23'39"	none	none	57	0	0%	4.577
48d	42°53'12"	82° 23'47"	P. millegrana	C. concolor	61	2	1%	4.577
48e	42°57'95"	82° 23'44"	P. millegrana	P. chloantha	63	2	1%	4.577
48f	42°52'85"	82° 23'32"	P. millegrana	P. chloantha	60	2	5%	4.577
48g	42°02'04"	82° 23'42"	P. millegrana	P. sulcata	56	3	5%	4.577
48h	42°56'93"	82° 23'44"	P. millegrana	P. sulcata	54	3	5%	4.577
49a	42°54'71"	82° 23'09"	P. millegrana	P. sulcata	57	7	50%	13.797
49b	42°57'96"	82° 23'10"	P. millegrana	P. sulcata	50	7	50%	13.797
49c	42°50'35"	82° 22'50"	P. millegrana	C. concolor	49	5	50%	13.797
49d	42°59'72"	82° 22'52"	P. millegrana	C. concolor	50	5	50%	13.797
49e	42°08'07"	82° 22'54"	P. millegrana	C. concolor	47	5	50%	13.797
49f	42°08'07"	82° 22'46"	P. millegrana	C. concolor	53	5	50%	13.797
49g	42°54'34"	82° 23'06"	P. millegrana	C. concolor	45	3	50%	13.797
49h	42°43'86"	82° 22'36"	P. millegrana	C. concolor	47	3	50%	13.797
50a	42°10'76"	82° 22'15"	P. millegrana	C. concolor	39	2	50%	7.153
50b	42°43'31"	82° 22'27"	P. millegrana	C. concolor	47	2	50%	7.153
50c	42°47'67"	82° 22'22"	P. millegrana	P. chloantha	39	2	25%	7.153
50d	42°04'83"	82° 22'19"	P. millegrana	P. chloantha	37	2	25%	7.153
50e	42°03'50"	82° 22'09"	P. millegrana	C. concolor	44	4	95%	7.153
50f	43°03'50"	82° 21'59"	P. millegrana	C. concolor	47	3	50%	7.153
50g	42°49'00"	82° 22'05"	P. millegrana	C. concolor	50	5	50%	7.153
50h	42°52'74"	82° 22'26"	P. millegrana	C. concolor	53	5	50%	7.153
51a	42°49'36"	82° 21'48"	P. millegrana	C. concolor	39	3	25%	8.679
51b	42°59'27"	82° 21'50"	P. millegrana	C. concolor	37	3	25%	8.679
51c	42°03'38"	82° 21'49"	P. millegrana	C. concolor	38	4	25%	8.679
51d	42°08'10"	82° 21'46"	P. millegrana	C. concolor	35	3	25%	8.679
51e	42°44'65"	82° 21'32"	P. millegrana	C. concolor	32	4	25%	8.679
52a	42°48'76"	82° 21'09"	P. millegrana	C. concolor	34	3	25%	3.969
52b	42°51'53"	82° 21'02"	P. millegrana	C. concolor	33	2	5%	3.969
52c	42°56'00"	82° 20'56"	P. millegrana	C. concolor	34	2	5%	3.969
52d	42°11'34"	82° 20'44"	P. millegrana	none	32	1	1%	3.969
52e	42°45'11"	82° 20'44"	none	none	35	0	0%	3.969
52f	42°50'67"	82° 20'44"	none	none	28	0	0%	3.969
53a	42°56'72"	82° 20'34"	none	none	32	0	0%	3.969
53b	42°52'49"	82° 20'30"	P. millegrana	none	31	1	1%	3.969
53c	42°57'20"	82° 20'34"	P. millegrana	none	35	1	1%	3.969
53d	42°43'66"	82° 20'26"	P. millegrana	none	26	1	5%	3.969
53e	42°53'44"	82° 20'13"	P. millegrana	none	23	1	5%	3.969
53f	42°11'11"	82° 20'03"	none	none	31	0	0%	3.969
53g	42°07'36"	82° 20'01"	P. millegrana	none	34	1	1%	3.969
54a	42°10'14"	82° 19'45"	P. millegrana	none	27	1	5%	3.969
54b	42°05'66"	82° 19'48"	P. millegrana	none	34	1	1%	3.969
54c	42°01'66"	82° 19'47"	P. millegrana	none	23	1	1%	3.969

55a	42°17'91"	82° 24'41"	P. millegrana	P. aipolia	39	3	25%	6.968
55b	42°42'74"	82° 24'40"	P. millegrana	P. aipolia	34	3	25%	6.968
55c	42°41'77"	82° 24'41"	P. millegrana	C. concolor	37	3	25%	6.968
55d	42°15'01"	82° 24'47"	P. millegrana	C. concolor	35	3	25%	6.968
55e	42°27'84"	82° 24'47"	P. millegrana	C. efflorescens	66	2	5%	6.968
55f	42°38'98"	82° 24'47"	P. millegrana	C. efflorescens	60	2	5%	6.968
55g	42°23'24"	82° 24'35"	P. millegrana	C. efflorescens	44	3	5%	6.968
55h	42°17'91"	82° 24'36"	P. millegrana	C. efflorescens	43	3	5%	6.968
56a	42°29'55"	82° 24'06"	P. millegrana	C. concolor	40	4	5%	9.995
56b	42°34'51"	82° 24'14"	P. millegrana	C. concolor	61	4	5%	9.995
56c	42°29'31"	82° 23'56"	P. millegrana	P. sulcata	42	4	25%	9.995
56d	42°20'47"	82° 24'14"	P. millegrana	C. concolor	38	3	25%	9.995
57a	42°25'80"	82° 23'46"	P. millegrana	C. concolor	42	2	5%	5.944
57b	42°31'49"	82° 23'37"	P. millegrana	C. concolor	44	2	5%	5.944
57c	42°38'50"	82° 23'37"	P. millegrana	C. concolor	40	2	5%	5.944
57d	42°38'14"	82° 23'44"	P. millegrana	C. concolor	43	3	5%	5.944
57e	42°27'01"	82° 23'20"	P. millegrana	C. concolor	34	3	5%	5.944
57f	42°23'63"	82° 23'18"	P. millegrana	C. concolor	44	3	5%	5.944
58a	42°33'42"	82° 23'11"	P. millegrana	P. chloantha	40	3	25%	6.968
58b	42°30'76"	82° 23'12"	P. millegrana	P. chloantha	37	3	25%	6.968
58c	42°35'60"	82° 23'10"	P. millegrana	C. concolor	42	6	50%	6.968
58d	42°25'69"	82° 23'10"	P. millegrana	C. concolor	41	3	25%	6.968
58e	42°36'08"	82° 22'36"	P. millegrana	C. concolor	48	3	25%	6.968
58f	42°41'53"	82° 22'36"	P. millegrana	C. concolor	45	3	25%	6.968
59a	42°39'35"	82° 22'26"	P. millegrana	P. aipolia	26	4	50%	11.424
59b	42°41'77"	82° 22'28"	P. millegrana	P. aipolia	28	4	25%	11.424
59c	42°33'18"	82° 21'55"	P. millegrana	C. concolor	41	4	50%	11.424
59d	42°37'65"	82° 21'55"	P. millegrana	C. concolor	39	4	25%	11.424
59e	42°41'27"	82° 21'55"	P. millegrana	P. aipolia	29	5	50%	11.424
59f	42°34'03"	82° 22'21"	P. millegrana	P. aipolia	25	4	25%	11.424
59g	42°32'21"	82° 22'12"	P. millegrana	P. aipolia	31	5	50%	11.424
59h	42°29'44"	82° 22'27"	P. millegrana	P. aipolia	28	4	50%	11.424
60a	42°27'14"	82° 21'35"	P. millegrana	P. aipolia	33	5	50%	10.699
60b	42°26'89"	82° 21'29"	P. millegrana	P. aipolia	20	4	25%	10.699
60c	42°26'41"	82° 21'20"	P. millegrana	P. aipolia	23	4	25%	10.699
60d	42°18'56"	82° 21'17"	P. millegrana	P. aipolia	27	5	25%	10.699
60e	42°35'47"	82° 21'47"	P. millegrana	P. sulcata	21	3	25%	10.699
60f	42°34'75"	82° 21'41"	P. millegrana	P. sulcata	23	3	25%	10.699
60g	42°23'51"	82° 21'47"	P. millegrana	C. concolor	62	4	75%	10.699
60h	42°21'70"	82° 21'44"	P. millegrana	C. concolor	60	4	75%	10.699
61a	42°17'84"	82° 21'10"	P. millegrana	C. concolor	52	2	50%	10.759
61b	42°26'04"	82° 20'37"	P. millegrana	C. concolor	50	2	50%	10.759
61c	42°24'95"	82° 20'40"	P. millegrana	P. aipolia	28	6	50%	10.759
61d	42°26'41"	82° 20'59"	P. millegrana	P. aipolia	31	6	50%	10.759
61e	42°25'80"	82° 21'09"	P. millegrana	P. chloantha	40	3	50%	10.759
61f	42°30'75"	82° 20'47"	P. millegrana	P. chloantha	48	4	50%	10.759

61g	42°30'50"	82° 20'53"	P. millegrana	P. chloantha	43	3	50%	10.759
61h	42°37'27"	82° 20'50"	P. millegrana	P. chloantha	44	4	50%	10.759
62a	42°26'88"	82° 20'26"	P. millegrana	C. concolor	49	4	75%	10.108
62b	42°24'46"	82° 20'16"	P. millegrana	C. concolor	48	4	75%	10.108
62c	42°28'57"	82° 20'17"	P. millegrana	C. concolor	53	4	75%	10.108
62d	42°32'07"	82° 20'23"	P. millegrana	C. concolor	50	4	75%	10.108
62e	42°23'85"	82° 20'05"	P. millegrana	C. concolor	49	4	75%	10.108
62f	42°18'54"	82° 20'04"	P. millegrana	C. concolor	34	4	75%	10.108
63a	42°23'24"	82° 19'40"	P. millegrana	C. concolor	45	4	75%	10.108
63b	42°23'72"	82° 19'36"	P. millegrana	C. concolor	27	4	75%	10.108
63c	42°25'78"	82° 19'53"	P. millegrana	C. concolor	44	4	75%	10.108
63d	42°21'31"	82° 19'52"	P. millegrana	C. concolor	34	4	75%	10.108

Appendix IV: Substrate pH Analysis Data

Bark samples were collected at 60 sites in Sarnia, 54 sites in Hamilton and 31 sites in Windsor. Lichen species richness was recorded for each tree from which the bark samples were collected. The pH of the bark samples was measured using the method from a study by Van Herk (2001). The site numbers are not related to the corresponding numbers from the initial and main surveys. The bark collection was independent from the other surveys. pH was measuring using a Corning pH meter.

Sarnia Sites	pH	Richness
1	5.5	4
2	5.2	2
3	6.0	4
4	5.2	2
5	6.2	6
6	5.5	3
7	5.7	6
8	5.8	5
9	5.3	3
10	5.4	3
11	5.3	5
12	5.4	3
13	5.8	6
14	5.3	3
15	5.4	4
16	5.6	4
17	5.4	3
18	6.1	7
19	6.0	6
20	5.5	3
21	5.5	6
22	5.2	2
23	6.0	4
24	5.7	4
25	5.6	3
26	5.1	3
27	5.5	5
28	4.5	2
29	4.6	1
30	5.0	1
31	5.7	2
32	5.0	7
33	4.9	6
34	5.4	5
35	5.5	1
36	5.4	3
37	5.5	4
38	5.7	2
39	5.4	4
40	5.7	3

Sarnia Sites	pH	Richness
41	5.8	1
42	5.7	2
43	5.7	3
44	5.4	5
45	5.7	3
46	5.8	0
47	5.9	2
48	6.1	2
49	5.7	1
50	5.4	2
51	5.7	1
52	4.9	6
53	5.3	4
54	5.5	3
55	5.5	4
56	5.7	2
57	6.0	2
58	6.1	1
59	5.7	1
60	5.9	3

Hamilton Sites	pH	Richness
1	6.1	0
2	5.2	2
3	6.4	4
4	5.8	5
5	6.1	5
6	5.8	5
7	5.9	0
8	6.0	3
9	5.9	2
10	5.7	3
11	5.4	5
12	6.1	5
13	6.1	3
14	6.0	4
15	5.9	4
16	6.3	3
17	6.4	3
18	6.2	5
19	5.4	3
20	6.2	4
21	5.4	4
22	6.1	5
23	5.5	5
24	5.9	3
25	6.1	3

Hamilton Sites	pH	Richness
26	5.6	4
27	6.3	4
28	6.1	5
29	5.9	2
30	5.0	3
31	5.0	4
32	6.6	4
33	5.2	3
34	6.0	3
35	6.1	3
36	5.9	5
37	5.7	2
38	5.7	5
39	6.0	3
40	6.0	0
41	5.8	2
42	5.6	2
43	6.3	5
44	5.9	4
45	6.1	0
46	5.7	5
47	6.1	5
48	5.0	4
49	5.3	4
50	5.7	2
51	6.0	1
52	5.7	0
53	5.7	0
54	5.6	2

Windsor Sites	pH	Richness
1	5.3	7
2	6.1	6
3	6.0	4
4	5.6	6
5	5.9	2
6	5.6	3
7	5.2	4
8	6.7	3
9	5.8	4
10	5.4	2
11	5.7	4
12	6.0	6
13	5.2	1
14	5.1	4
15	5.4	3
16	5.7	4

Windsor Sites	pH	Richness
17	5.9	3
18	5.3	4
19	5.3	3
20	5.2	5
21	5.7	3
22	5.3	2
23	5.4	4
24	5.4	3
25	5.7	5
26	5.3	4
27	5.2	5
28	5.4	2
29	5.8	3
30	5.5	2
31	5.7	1

Appendix V: Correlations

The lichen community and habitat variables collected during the main survey were analyzed using statistics in order to determine whether any relationship existed between any two variables. Sites were divided into two categories; `clean` and `dirty` depending on their proximity to any know source of contamination. The statistical analysis was done using SPSS. The pH of the bark samples was measured using the method from a study by Van Herk (2001). Diameter refers to the diameter of the surveyed tree at chest height. Canopy The distance from the lake and river was calculated using a topographic map to estimate the straight-line distance of the site to the nearest body of water. The road category variable relates to Table 3.1. This data was collected during the main survey. The Index of Atmospheric Purity (IAP) uses the presence, absence and abundance of lichens in order to generate an air quality index.

Sarnia		pH	Richness	IAP	Diameter	IHI	Lake	River	Road Category	Distance Road	% Cover
pH	All	1	0.02	-0.2	0.08	0.18	-0.21	-.32*	-.33**	-0.22	-.46**
	Clean	1	-.72**	-0.15	0.11	0.3	.48**	-0.12	-0.33	-0.11	0.24
	Dirty	1	.80**	-.40*	-0.02	0.23	-.49**	-.36*	-0.15	-0.35	-.68**
Richness	All	0.02	1	0	0.14	0.04	-0.19	-0.18	0.14	0.1	-0.06
	Clean	-.72**	1	0.08	0.05	-0.25	-0.31	0.2	0.36	0.15	-0.07
	Dirty	.80*	1	-0.15	0.11	0.25	-.41*	-.48**	-0.24	-0.25	-.52**
IAP	All	-0.2	0	1	-.26*	-0.16	-0.01	-0.17	0.03	0.12	.42**
	Clean	-0.15	0.08	1	-.37*	-0.02	-.36*	-.52**	-0.07	-0.09	0.32
	Dirty	-.40*	-0.15	1	0	-0.23	0.31	0.12	0.07	0.12	.47**
Diameter	All	0.08	0.14	-3E-1*	1	0.07	0.14	-0.22	0.1	-0.09	0.04
	Clean	0.11	0.05	-.37*	1	-0.08	0.2	-0.15	0.08	0.04	-0.14
	Dirty	-0.02	0.11	0	1	0.15	0.07	-0.22	0.3	-0.3	0.35
IHI	All	0.18	0.04	-0.16	0.07	1	.38**	-4E-1**	-0.08	-0.25	0.07
	Clean	0.3	-0.25	-0.02	-0.08	1	.52**	-.42*	-0.05	-0.26	0.2
	Dirty	0.23	0.25	-0.23	0.15	1	0.27	-.38*	-0.15	-0.16	0.057
Lake	All	-0.21	-0.19	-0.01	0.14	.38**	1	0.15	0.01	0.01	.38**
	Clean	.48**	-0.31	-.36*	0.2	.52**	1	0.07	-0.2	-0.09	0.27
	Dirty	-.49**	-.41*	0.31	0.07	0.27	1	0.23	0.24	0.12	.46*
River	All	-3E-1*	-0.18	-0.17	-0.22	-4E-1**	0.15	1	0.06	0.21	0.06
	Clean	-0.12	0.2	-.52**	-0.15	-.42*	0.07	1	-0.07	0.16	-0.08

	Dirty	-.36*	-.48**	0.12	-0.22	-.38*	0.23	1	0.15	0.26	0.12
Road Category	All	-3E-1**	0.14	0.03	0.1	-0.08	0.01	0.06	1	0.06	-0.09
	Clean	-0.33	0.36	-0.07	0.08	0.05	-0.2	-0.07	1	0.01	-0.24
	Dirty	-0.15	-0.24	0.07	0.3	-0.15	0.24	0.15	1	0.08	-0.02
Distance Road	All	-0.22	0.1	0.12	-0.09	-0.25	0.01	0.21	0.06	1	.27*
	Clean	-0.11	0.15	0.09	0.04	-0.26	-0.09	0.16	0.01	1	.47**
	Dirty	-0.35	-0.25	0.12	-0.3	-0.16	0.12	0.26	0.08	1	0.05
% Cover	All	-4E-1**	-0.06	.42**	0.04	0.07	.38**	0.06	-0.09	.27*	1
	Clean	-0.24	-0.07	-0.32	-0.14	0.2	0.27	-0.08	-0.24	.47**	1
	Dirty	-.68**	-.53**	.47**	0.35	0.06	.46*	0.12	-0.02	0.05	1

The correlations for all measured variables for all sites in Sarnia, 'dirty' and 'clean' sites. Highlighted cells indicate variables found to have significant correlations at either the 0.05 or 0.01 level. * represents a correlation that is significant at the 0.05 level while ** signifies a correlation that is significant at the 0.01 level.

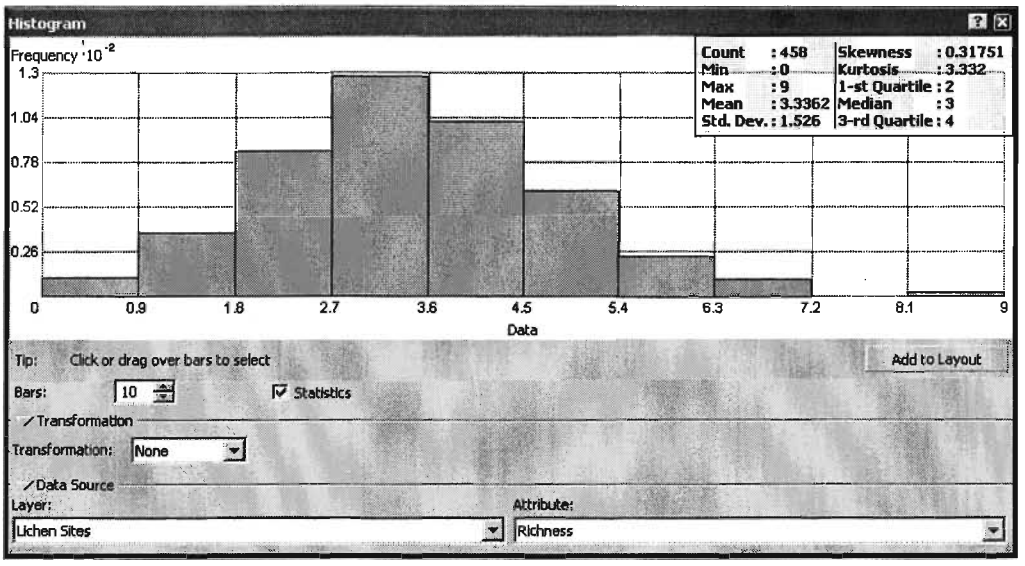
Hamilton		pH	Richness	Diameter	Road	Distance road	% Cover
pH	All	1	0.03	0.2	0.15	-0.17	0.02
	Clean	1	0.17	0.01	0.13	0.25	0.15
	Dirty	1	-0.09	0.24	0.29	-.65**	-0.15
Richness	All	0.03	1	0.01	0.04	-.28*	.71**
	Clean	0.17	1	-.52**	.62**	-0.19	.48**
	Dirty	-0.09	1	0.21	0.23	-0.18	.70**
Diameter	All	0.1	0.01	1	-.57**	-0.15	0.11
	Clean	0.01	-.52**	1	-.86**	-0.31	-0.03
	Dirty	0.24	0.21	1	-0.16	-0.12	0.15
Road	All	0.15	0.04	-.57**	1	-0.06	-0.02
	Clean	0.13	.62**	-.89**	1	.41*	0.16
	Dirty	0.29	0.23	-0.16	1	-0.23	0.11
Distance road	All	-0.17	-.28*	-0.15	-0.06	1	-.30*
	Clean	-0.25	-0.19	-0.31	.41*	1	-0.25

	Dirty	-.65**	-0.18	-0.12	-0.23	1	-0.29
%Cover	All	0.02	.71**	0.11	-0.02	-.30*	1
	Clean	0.15	.48**	-0.03	0.16	-0.25	1
	Dirty	-0.15	.70**	0.15	0.11	-0.29	1

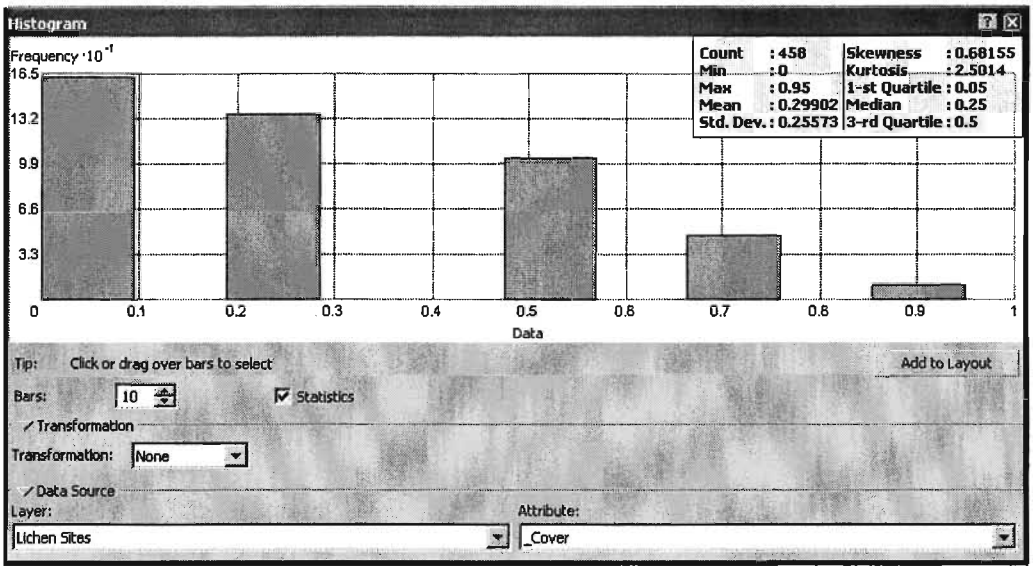
The correlations for all measured variables for all sites in Hamilton, 'dirty' and 'clean' sites. Highlighted cells indicate variables found to have significant correlations at either the 0.05 or 0.01 level. * represents a correlation that is significant at the 0.05 level while ** signifies a correlation that is significant at the 0.01 level.

Appendix VI
Histograms

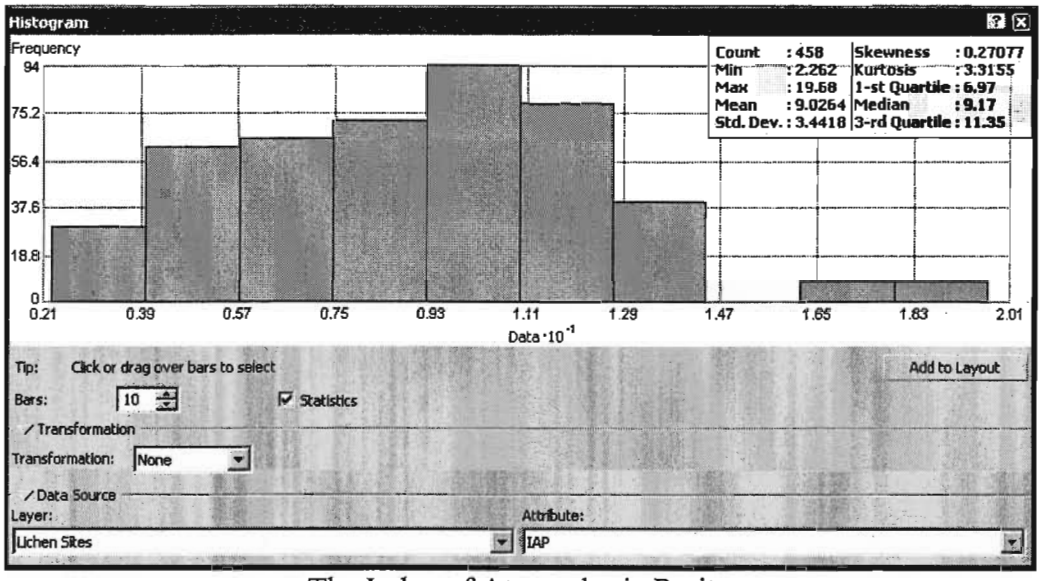
Geographic Information Systems (GIS) was used to examine any spatial relationships between lichen community and habitat variables. Arc GIS was used to produce the maps. The histograms which were used to determine the categories of data are below.



Lichen Species Richness



Percent Cover



The Index of Atmospheric Purity

Appendix VII
Re-testing of Samples to Determine Error of pH Meter

In order to assess the degree of error of the pH data, the pH samples were tested for pH twice for each sample. The pH probe was rinsed with deionized water between each test. The probe was left in each sample for fifteen seconds in order to allow time for it to stabilize. The standard deviation was calculated in order to assess the difference between the

Sarnia	pH Test 1	pH Test 2	Std.
s1	5.5	5.8	0.21
s2	5.2	4.7	0.33
s3	6.0	5.8	0.13
s4	5.1	5.6	0.31
s5	6.1	5.7	0.26
s6	5.5	5.2	0.21
s7	5.7	5.1	0.45
s8	5.8	5.3	0.33
s9	5.3	5.6	0.19
s10	5.4	4.7	0.49
s11	5.3	5.8	0.34
s12	5.4	5.1	0.19
s13	5.8	6.1	0.16
s14	5.3	4.1	0.38
s15	5.4	5.5	0.08
s16	5.6	5.9	0.26
s17	5.4	5.0	0.31
s18	6.1	5.7	0.26
s19	6	5.6	0.26
s20	5.5	5.1	0.27
s21	5.5	5.2	0.25
s22	5.2	4.6	0.38
s23	6	5.9	0.07
s24	5.7	5.3	0.31
s25	5.6	5.3	0.18
s26	5.1	5.4	0.19
s27	5.5	5.3	0.12
s28	4.5	4.3	0.15
s29	4.6	4.9	0.17
s30	5	5.3	0.24
s31	5.7	6	0.16
s32	5	4.3	0.45
s33	4.9	4.3	0.43
s34	5.4	5.2	0.18
s35	5.5	5.7	0.12
s36	5.4	5.2	0.19
s37	5.5	5.7	0.14
s38	5.7	5.9	0.07
s39	5.4	5.6	0.18
s40	5.7	6	0.14

s41	5.8	5.9	0.05
s42	5.7	5.1	0.41
s43	5.7	5.5	0.14
s44	5.4	5.6	0.12
s45	5.7	6	0.18
s46	5.8	5.3	0.35
s47	5.9	5.5	0.27
Sarnia	pH	Test 2	Std.
s48	6.1	5.7	0.29
s49	5.7	5.3	0.28
s50	5.4	5.5	0.09
s51	5.7	5.3	0.28
s52	4.9	5.4	0.31
s53	5.3	5.7	0.26
s54	5.5	5.9	0.26
s55	5.5	5.1	0.33
s56	5.7	5.8	0.08
s57	6	5.7	0.19
s58	6.1	5.7	0.26
s59	5.7	5.4	0.23
s60	5.9	5.4	0.31